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THE FORMATION OF BIREFRINGENT CRYSTALS IN THE SUPRARENAL CORTEX

By J. M. YOFFEY and J. S. BAXTER
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INTRODUCTION

Kaiserling in 1898 (quoted by Kaiserling & Orgler, 1902) first noted the presence of birefringent material in the suprarenal cortex. Albrecht & Weltmann (1911) considered that the birefringent substances were cholesterol esters, and emphasized that they formed doubly refracting droplets, as opposed to the crystals which were formed by tripalmitin, tristearin, triolein and fatty acids. They worked with frozen sections after formalin fixation. In the human suprarenal they found abundant spherical crystals, but could not demonstrate them in guinea-pig and rabbit suprarenals without a special technique (see also Weltmann, 1913) involving treatment of the section with methyl alcohol, and with a saturated solution of green soap in methyl alcohol, as a result of which they thought the lipoids underwent saponification. They gave no illustrations. Landau & McNee (1914) observed birefringent material in the suprarenal of rabbits, and believed they could establish a relationship between the amount of such material and the cholesterol content.

Bennett (1940) noted birefringent crystals in the adrenal cortex of the cat (formol fixation) 'scattered...throughout the secretory zone'. Applying the digitonin test of Leulier & Revol (1930) he found that 'sections of glands treated with alcoholic or aqueous digitonin after fixation display an abundance of birefringent crystals in this zone'. He believed that these crystals were 'probably for the most part due to the presence of cholesterol', but also observed that crystals of desoxycorticosterone acetate were birefringent, the inference presumably being that some of the crystals found in the gland might be corticosterones or closely related compounds. He does not appear to have investigated the crystals in detail, and gave one figure (fig. 7 of his paper) at a magnification of $\times 67$.

Weaver & Nelson (1943) made a special study of birefringent material in the suprarenal cortex of the rat, and investigated the effect upon it of pituitary adrenotropic hormone. Their work was done at low magnification ($\times 45$), on frozen sections made after formalin fixation and embedding in gelatin. They distinguished four zones in the normal rat. The most striking was the main optically active zone, corresponding to most of the zona fasciculata, and a much narrower optically active zone corresponding to the zona glomerulosa. Between these two they described a narrow subglomerular optically inactive zone, while

* Aided by a grant from the Colston Research Fund.

between the main optically active zone and the medulla—i.e. in the zona reticularis—was an inner zone with only scattered birefringent crystals.

In view of the possible light which a closer study of these crystals might throw on the activity of the suprarenal cortex, it was decided to investigate their formation and distribution in greater detail.

MATERIAL AND METHODS

The work has been done mainly on the rat and cat, but sheep suprarenals have been examined also, and one specimen each of the suprarenal of horse, chimpanzee and man. In the cat and rat, glands were removed immediately after death, in the sheep 3–4 hr. after death. The human suprarenal was from a man aged forty-five who died of acute coronary thrombosis, and it was obtained at post-mortem examination 5 hr. after death; the suprarenals appeared to be quite normal, and cell preservation was excellent. The suprarenals of a pregnant female chimpanzee were obtained 18 hr. after death, and of a castrated male horse immediately after death.

Sections were in all cases made with the freezing microtome, using both unfixed material, as well as glands fixed in 10 % neutral formol-saline with or without subsequent embedding in gelatin. Sections were cut at thicknesses ranging from 5 to 50 μ, and were mounted either in normal saline, distilled water, glycerine, syrup of laevulose, or glychrogel (Zwemer, 1933). Where a volatile mounting medium was used, the edge of the coverslip was ringed with paraffin wax or vaseline.

For the detection of birefringent crystals, the examination of unstained sections with polarized light is quite adequate. Some sections were stained with Sudan III according to the technique of Romeis (1929), in order to study the relationship between the birefringent crystals and sudanophil material; other sections were stained with haematoxylin. The Schultze reaction (Lison, 1936) and the digitonin reaction (Leulier & Revol, 1930) were also studied.

For obtaining the phenylhydrazine reaction for corticosterones, Pechet's (1946) (quoted without details by Dempsey & Wislocki, 1946) modification of Bennett's (1940) original method was employed, as follows: Formalin-fixed sections cut with the freezing microtome are immersed in a saturated solution of 2, 4-dinitrophenylhydrazine sulphate in 50 % alcohol, contained in a covered glass capsule and kept overnight in an incubator at 37° C. The sections are then washed in several changes of distilled water for about an hour and mounted in glychrogel. A positive reaction is indicated by the development of a deep orange-red colour.

RESULTS

In the normal suprarenal of the rat, both adult male and female, a frozen section of a fresh unfixed gland mounted in normal saline, syrup of laevulose, or distilled water shows no crystalline birefringence but only the weak fibrillar birefringence of the capsule, fibrous septa and walls of the larger blood vessels

(Pl. 1, figs. 1, 2). In the horse this fibrillar birefringence is much more in evidence because of the thicker capsule and the presence of numerous well-marked fibrous septa extending from the capsule into the gland, but there is equally a complete absence of birefringence in the cortical cells themselves. In the cat (Pl. 2, figs. 5, 6) there may be a few birefringent crystals in unfixed frozen sections; there is sometimes present, very characteristically, in the outer part of the zona fasciculata a weak diffuse birefringence which is perhaps best described as a luminescence. In the inner part of the fasciculata occasional globules may be seen, but they are not birefringent. In man (Pl. 3, fig. 15) the luminescence of the fasciculata appears to be well marked.

If the sections are kept at room temperature and frequently examined for a few days after mounting, birefringent crystals gradually appear (Pl. 1, figs. 2–4). In the cat they sometimes appear much more rapidly than in the rat, and in fact may begin to be visible in the zona fasciculata within an hour or two after mounting, progressively replacing to an increasing extent the original luminescence, until the outer part of the zona fasciculata is full of crystals. Occasionally, as already noted, a few crystals may even be present immediately after mounting. In the human suprarenal examined, the luminescence was much more stable, and even after 4 weeks had resolved into crystals only at scattered points. The crystals in all the species are preponderantly acicular. But sometimes in the deeper part of the fasciculata there may be seen spherical crystals showing the typical black cross of polarization.

Formalin-fixed and gelatin-embedded sections show a maximum concentration of crystals (Pl. 2, fig. 7). These are evident to a varying extent in the zona glomerulosa, in addition to their main concentration in the zona fasciculata. The crystals in the zona glomerulosa appear as a rule to be finer and more granular than those in the fasciculata. In whatever zone they occur, the crystals can always be seen in thin sections to be located within the cells (Pl. 3, fig. 10).

Zoning. In a fresh frozen section of the rat suprarenal observed by transmitted light immediately after mounting, zoning is quite evident (Pl. 1, fig. 1). The glomerulosa stands out, because there is a clear sub-glomerular zone separating it from the fasciculata. Furthermore, the glomerulosa is darker than the fasciculata, and its texture appears to be different.

Effect of mounting medium. The sections were kept in daylight at room temperature, and under these circumstances the crystals begin to form and gradually increase until a maximum is reached. An obvious possibility to be considered is that they are derived from the mounting medium, particularly in the case of laevulose. As far as the saline mounts are concerned, the crystals of sodium chloride are isotropic, and their shape is quite characteristic.

To obviate all possibility of crystalline contaminants, some sections were mounted in distilled water. This is not an ideal mounting medium for unfixed cells, but at any rate one can be sure that the crystals which form after its use are not extraneous. Though we are not reporting here in detail on the optical properties of the crystals, it is perhaps worth noting that in respect to these

properties the crystals which appear after the use of the various mounting media are all alike.

The distribution of the crystals is in some ways typical for the various mountants. Frequently there are two types present, namely, larger aggregates, and finer dispersed crystals. In the sections mounted in laevulose, more especially during the first day or so after mounting, the fine crystals are obviously within the cells of the zona fasciculata, and the columns of these crystal-containing cells are readily identified. But frequently after a few days the crystals rise to the surface of the section, immediately under the coverslip, and are dispersed evenly without any obvious relation to the cortical cells, suggesting that the latter are disintegrating. In spite of this the crystals still show some degree of zoning, and it is unlikely that they are moving about very freely on the surface of the section. Thus in the normal gland they are always in the cortex, never in the medulla (Pl. 1, fig. 4; Pl. 2, fig. 7; Pl. 3, fig. 11); and even in the cortex some further zoning is usually evident, the crystals being most concentrated in the outer portion of the zona fasciculata, with a more variable distribution in the zona glomerulosa.

The sections of the human suprarenal (Pl. 3, figs. 11, 15) illustrate well the combined effects of formalin fixation, embedding in gelatin, and mounting in glychrogel. Pl. 3, fig. 15, from an unfixed section mounted in normal saline, shows the diffuse luminescence already mentioned; Pl. 3, fig. 11, shows by contrast a very rich crop of crystals.

DISCUSSION

Are the crystals a breakdown product of a more complex precursor? The findings suggest the possibility that the birefringent crystals are formed by the breakdown of a non-birefringent precursor occurring in the fresh gland tissue. This might conceivably be a lipoprotein, and if this were so there is the possibility that the cortical hormones enter the blood as lipoproteins, since these are known to constitute about one-fourth of the blood-plasma proteins (Cohn, 1946). The breakdown of a lipoprotein precursor, in sections mounted in normal saline or distilled water, could be attributed to the presence of proteolytic enzymes, with whose action formol might be expected to interfere. Hoerr's (1936) finding in the guinea-pig, that 'frozen sections of fresh adrenals usually show more bi-refractile droplets than formalin-fixed material' would seem to fit in very well with this idea. But in the present experiments the findings have as a rule been diametrically opposed to those of Hoerr, and far more crystals have been present in formalin-fixed than unfixed sections, the latter when absolutely fresh having no crystals whatever in the rat (see Pl. 1, fig. 2).

Are the crystals cholesterol? Albrecht & Weltmann (1911), Landau & McNee (1914), Hoerr (1936), Bennett (1940), and many other workers have concluded that the birefringent crystals consisted of cholesterol and cholesterol esters. Romeis (1932) was of the opinion that cholesterol esters occurred in unfixed material as birefringent droplets, which only became crystalline after formal

fixation. This does not accord with our findings, since in the unfixed frozen sections no birefringent material in the form of either droplets or crystals has been present. If the crystals which do finally appear are cholesterol, then the implication is either that this cholesterol was to begin with part of a more complex molecule which was not crystalline and birefringent or that it can also be present in a non-crystalline and frequently non-birefringent form. That this latter is quite possible is suggested by the fact that, after crystals have appeared in formol-fixed material, an increased formation of crystals follows the application of digitonin (Pl. 2, figs. 8, 9).

Cholesterol may not always be visible in birefringent form, even though known to be present, because it may be in solution in fatty substances. Considerable amounts of cholesterol may be dissolved in fat without exhibiting any birefringence. Thus Okey found (1944) that the livers of guinea-pigs fed with large amounts of cholesterol might contain greatly increased amounts both of fatty acids (17·0–24·1 % against a normal range of 2·6–9·0 %) and of total cholesterol (3·5–5·7 % as against a normal range of 0·30–0·83 %). Nevertheless, 'Efforts to determine the localization of the cholesterol and to distinguish it from other liver lipoids by means of the petrographic microscope were unsuccessful. This may have been because the cholesterol was in solution in the fat and the solution was not birefringent.'

The Schultze reaction (Lison, 1936) was strongly positive in the outer part of the fasciculata and to a lesser extent in the zona glomerulosa in all the sections studied (Pl. 4, fig. 19).

In the sheep suprarenal crystals were not so plentiful, nor as a rule did they appear as readily in unfixed sections, as in the cat and rat. According to Beumer (1914), 1·2 % of the dried substance of the sheep suprarenal is free cholesterol and 0·2 % is cholesterol esters. Leulier & Revol (1930) give a considerably higher figure for the total cholesterol, namely, 2·12–4·06 % in the cortex, 3·45–6·60 % in the medulla. The high medullary cholesterol is especially interesting in view of the non-formation of crystals in the medulla.

Free fatty acids form 1·3 % of the sheep suprarenals (Beumer, 1914).

Birefringent crystals and sudanophilic substances. The birefringent and sudanophil zones are closely related, but do not completely coincide with one another. Pl. 4, figs. 16, 17, from the chimpanzee, illustrate this very well. The crystalline and sudanophil zones are not sharply demarcated from one another, and do in fact intermingle, but the richest accumulation of crystals is in the glomerulosa and juxta-glomerular zone, while the richest accumulation of sudanophil material is deep to this. A high-power view of this section (Pl. 4, fig. 17, ×480) shows that the sudanophil and crystalline material may occur together in the same cell, but the crystals themselves are never sudanophil.

Birefringent crystals and corticosterones. The distribution of the crystals also closely follows the zone in which the phenylhydrazine reaction occurs (Bennett, 1940), or rather what is believed to be Pechet's modification thereof (Dempsey & Wislocki, 1946). The specificity of the phenylhydrazine reaction for cortico-

sterones has been questioned (e.g. Albert & Leblond, 1946), but if Bennett's (1940) assumption is correct, then this reaction, most markedly positive in the outer fasciculata (Pl. 4, fig. 18), would indicate that the birefringent crystals are most numerous in the region where the biologically active adrenal cortical sterones are present.

In an attempt to throw further light on this question, Dr S. H. Piper and Mr H. J. Welland, of the Department of Physics at Bristol University, have been kind enough to help us by applying X-ray crystallographic methods. The full results will be presented elsewhere, but from the point of view of the problem under consideration it is worth noting that the crystal spacings obtained in powder photographs were not the same as those obtained with crystals of pure desoxycorticosterone acetate, desoxycorticosterone, androsterone and androstane, ascorbic acid, tripalmitin, or tristearin, or cholesterol in the crystalline forms of the latter with which comparison was made. This must not be taken to prove that the crystals are not cholesterol, for this substance can crystallize out in a number of different forms.

SUMMARY

1. In frozen sections of the fresh and unfixed suprarenal glands of the adult albino rat, male and female, there is no birefringent material in the cortex proper, though there is birefringence in the capsule.

2. When the sections are mounted in either distilled water, normal saline, or syrup of laevulose, birefringent material gradually appears in the cortex, mainly in the zona fasciculata, for the most part in the form of acicular crystals, but in the deeper part of the fasciculata occasional spherical crystals with the black cross of polarization may be seen.

3. In sections fixed in formalin, embedded in gelatin and mounted in glychrogel abundant crystals are found throughout the cortex, but are most numerous in the zona glomerulosa and the outer part of the zona fasciculata.

4. The Schultze reaction for cholesterol is strongly positive in the outer part of the zona fasciculata and in the zona glomerulosa.

5. In the cat the same observations have been made, except that there may be a diffuse luminescent birefringence from the outset, and the formation of crystals may take place more quickly than in the rat.

6. X-ray powder photographs of the cat suprarenal cortex have failed to establish the identity of the crystals with desoxycorticosterone, androsterone, androstane, desoxycorticosterone acetate, ascorbic acid, tripalmitin, tristearin, or with cholesterol in the crystalline form of the latter which was employed.

7. The crystals are intracellular, and usually closely associated in distribution with sudanophil non-crystalline material. The crystals themselves are not sudanophil.

8. In the region of maximal crystal formation, namely the outer portion of the zona fasciculata, there is a strongly positive phenylhydrazine reaction for corticosterones.

9. Some observations are reported on one case each of a castrated male horse, a pregnant female chimpanzee, and a man aged 45 years.

We are greatly indebted to Prof. T. Reichstein of the University of Basel for kindly supplying us with pure specimens of desoxycorticosterone, androsterone and androstane, to Professor Wilson Baker, Dr Thomas Malkin and Dr G. T. Young of the Chemistry Department for their advice on chemical problems, to Dr S. H. Piper and Mr H. J. Wellard of the Physics Department for their crystal photographs, to Mr I. S. Loupekine of the Geology Department for examination of the optical properties of crystals, and to Mr J. E. Hancock for the photomicrographs.

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EXPLANATION OF PLATES

Plate 1

Adult male rat. Fresh frozen section of the adrenal, cut at 50μ and mounted in syrup of laevulose. Fig. 1. Photographed by transmitted light immediately after cutting, $\times 60$ (the black ringed areas are air-bells). Fig. 2. The same section photographed in polarized light. There is a little fibrillar birefringence in the medulla, and a piece of peri-adrenal fat shows up brightly. Fig. 3 shows the same field after 48 hr. Some coarse birefringent crystals have formed in the outer part of the cortex and there is a faint background of dust-like material. Fig. 4. After 5 days both these crystal forms have increased. The fine crystalline material outlines the zona glomerulosa and the outer part of the zona fasciculata, the radial cell columns of the latter being very evident. A narrow optically inactive zone is also present between them.

Plate 2

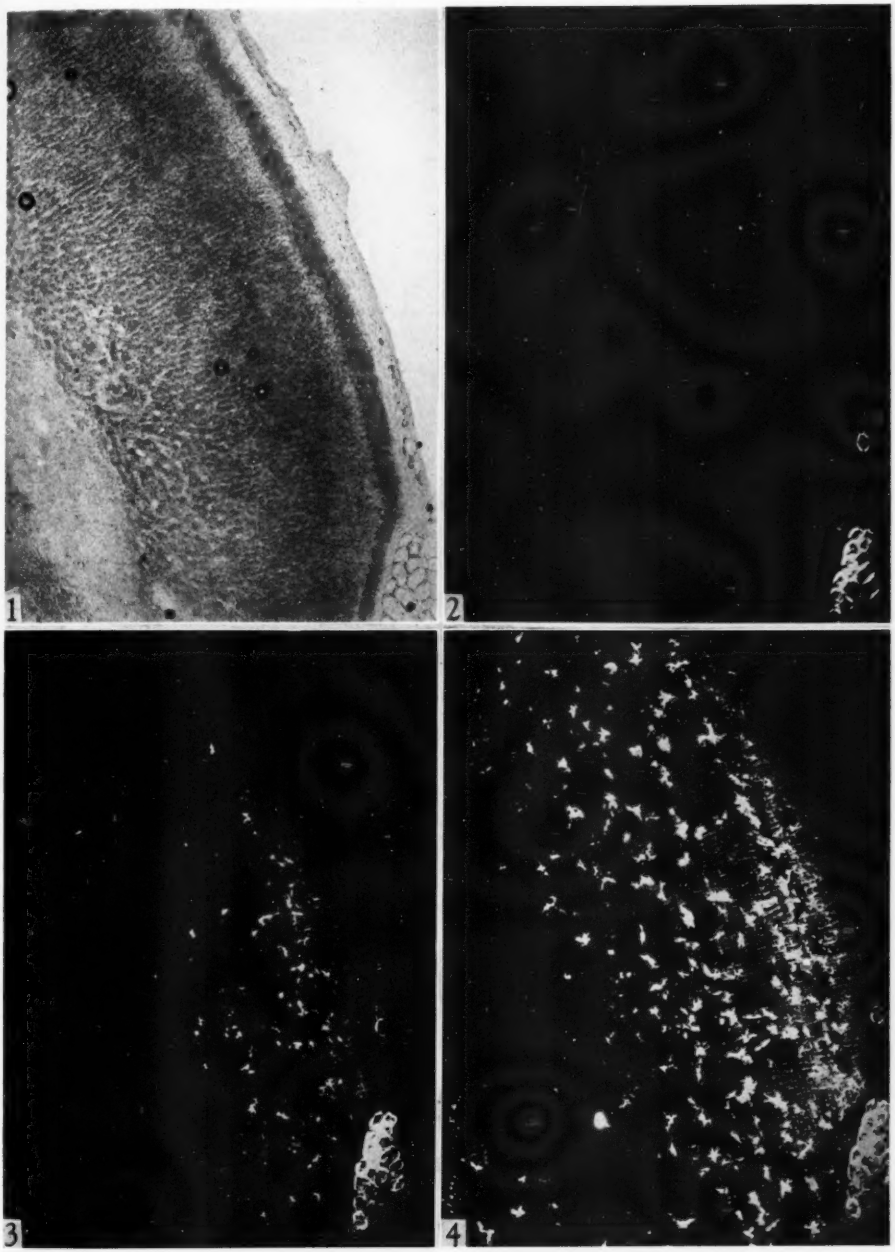
Figs. 5 and 6 show fresh, frozen sections at 50μ of the adrenal cortex ($\times 60$) from an 11-month male cat. The outer half of the zona fasciculata appears very dense with transmitted light (fig. 5); some fine birefringent material is seen here with polarized light (fig. 6). Fig. 8 is a part of the zona fasciculata of this adrenal viewed with polarized light at a higher magnification ($\times 200$). Fig. 9 shows the same field after treatment with alcoholic digitonin (Leulier-Revol test for free cholesterol). The amount of birefringent crystalline material has greatly increased. During the incidental manipulations the section has been reversed so that the field shown in Fig. 9 is a mirror image of Fig. 8. Fig. 7 is a photomicrograph, $\times 60$, in polarized light of a 50μ section of the adrenal of an adult female rat, fixed in 10% formol and embedded in gelatin. Note the density of the crystals, their fine dispersion, and their tendency to be arranged in zones. The medulla is at the bottom of the plate.

Plate 3

Fig. 10. 5μ frozen section through the zona fasciculata of the cat adrenal (formol fixation), stained with haematoxylin and photographed in polarized light, $\times 400$. All nuclei are dark; birefringent material (bright) is intracellular. Fig. 11. 5μ frozen section of a human suprarenal (formol fixation, gelatin embedding) photographed with polarized light, $\times 31$. *C.M.J.* cortico-medullary junction. Birefringent material in crystalline form fills the cells of the outer fasciculata. Figs. 12, 13. 50μ fresh frozen section of the adrenal from a chimpanzee; fig. 12 is photographed in transmitted light, and fig. 13 in polarized light, $\times 60$. Fibrillar birefringence is seen in the capsule while the spongiocyte zone shows luminescence. Figs. 14, 15. Similar photomicrographs ($\times 31$) of a 50μ fresh frozen section of the adrenal of a man aged 45 years. Note the very pronounced luminescence of the fascicular zone in fig. 15 and contrast with the crystalline birefringence in the same zone of the human adrenal shown in fig. 11.

Plate 4

Fig. 16. Photomicrograph ($\times 31$) of a section of the chimpanzee adrenal to show the distribution of the sudanophil and birefringent material. The greatest intensity of birefringent material by no means corresponds with the deepest staining with Sudan III. Fig. 17. High-power view ($\times 480$) of the same section to show the boundary zone between zona glomerulosa on the left, and zona fasciculata on the right. The birefringent crystals are *not* sudanophil, and the degree of sudanophilia is not indicative of the amount of birefringent material present. Fig. 18. Photomicrograph ($\times 53$) of the dinitrophenylhydrazine reaction in the adrenal cortex of a chimpanzee (50μ frozen section, formol fixation without embedding in gelatin). Fig. 19. Photomicrograph ($\times 38$) of the Schultze reaction for cholesterol in the human adrenal.



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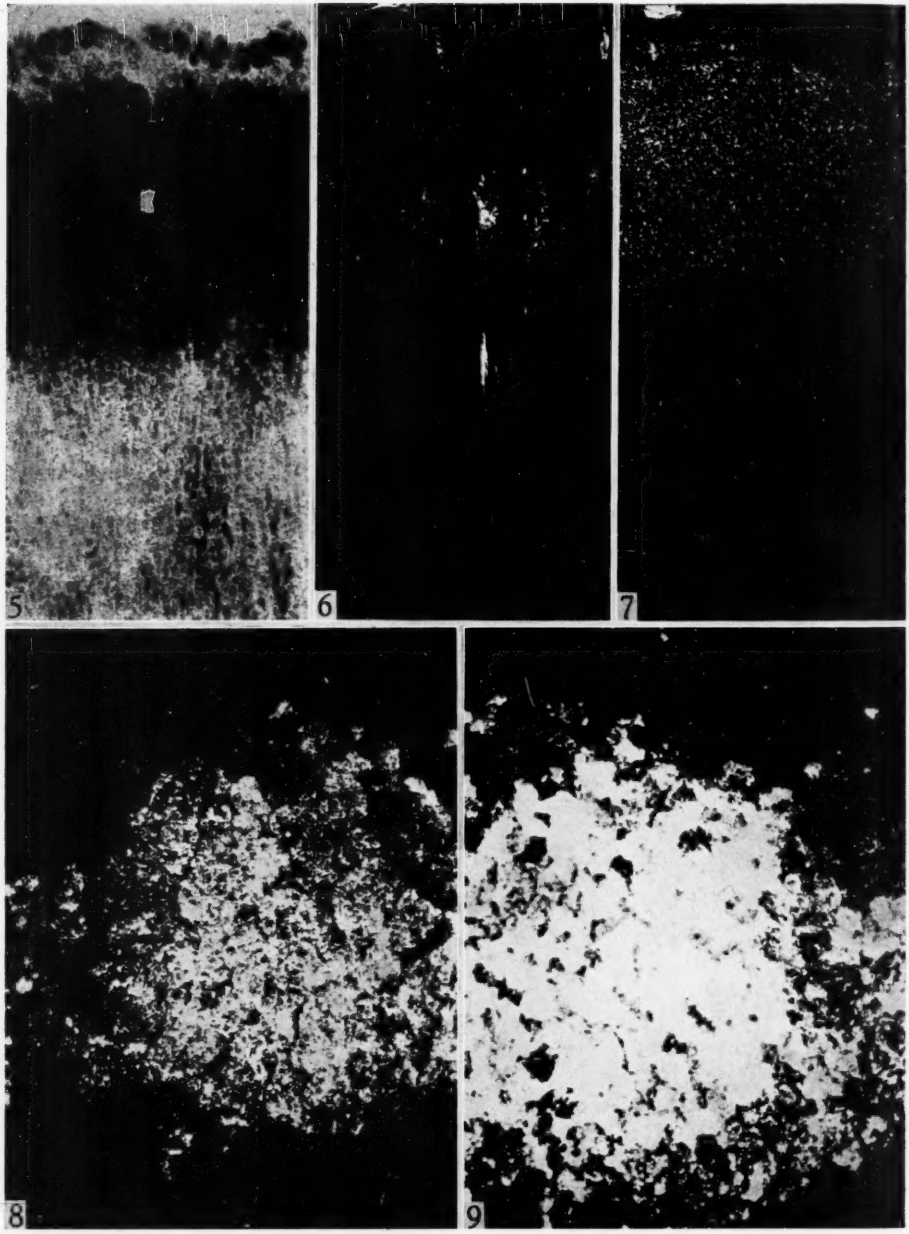
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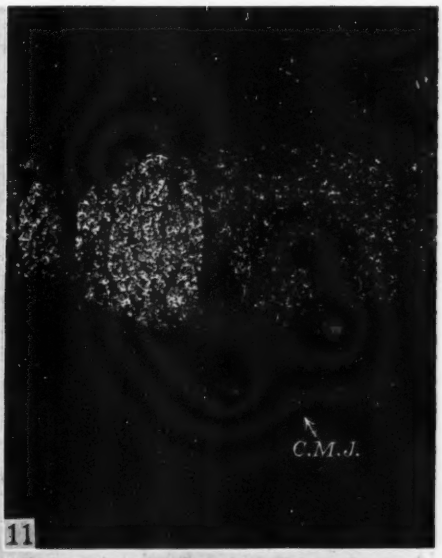

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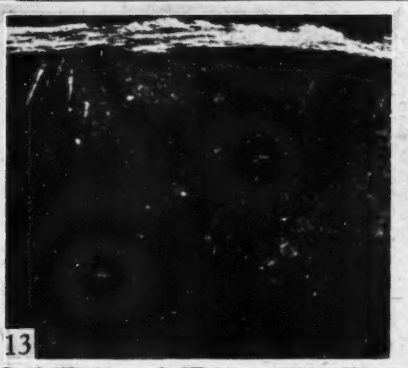

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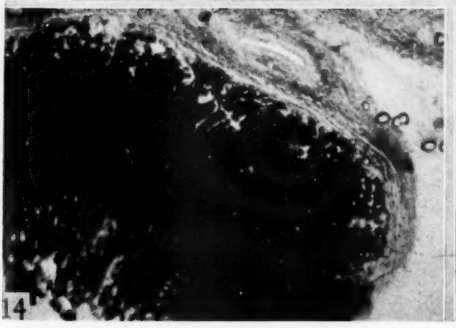

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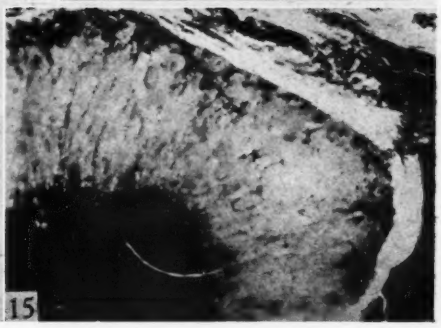

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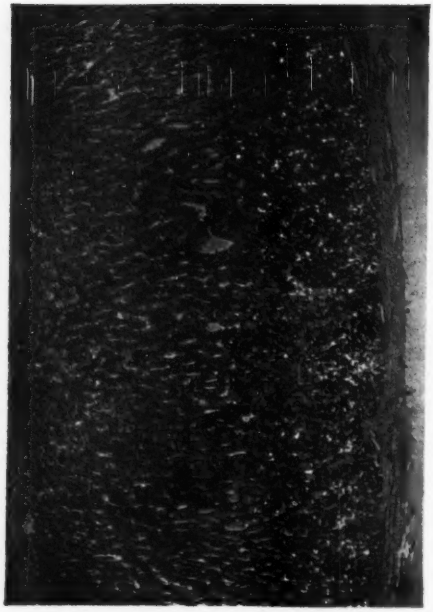
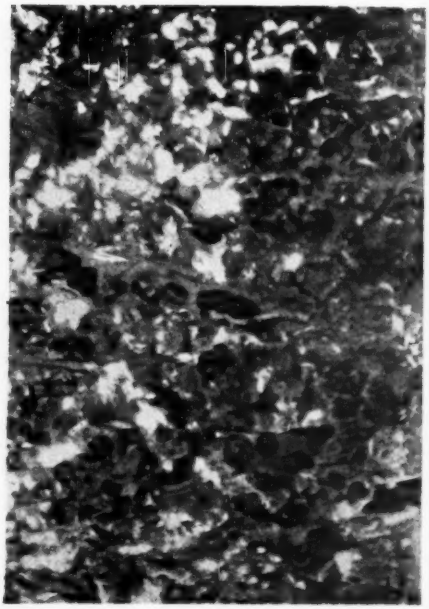


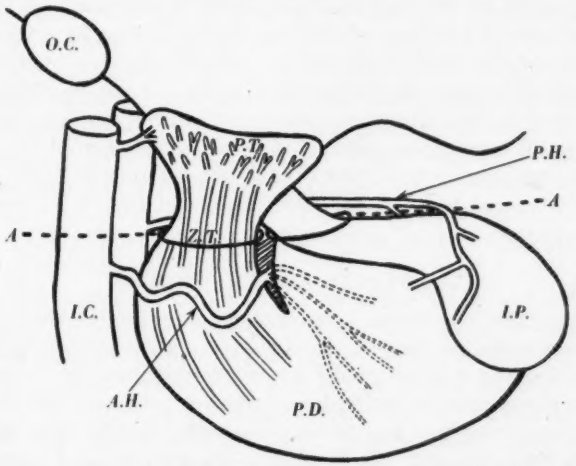


YOFFEY AND BAXTER—BIREFRINGENT CRYSTALS IN THE SUPRARENAL CORTEX

THE BLOOD VESSELS OF THE RABBIT'S PITUITARY GLAND, AND THE SIGNIFICANCE OF THE PARS AND ZONA TUBERALIS

By G. W. HARRIS, *Anatomy School, University of Cambridge*

In 1937 Dawson described a specialized region of the anterior lobe of the pituitary gland in the rabbit and cat, which he termed the zona tuberalis. This structure lies at the anterior pole of the gland, partially separated from the main mass of the pars distalis by connective tissue. It merges dorsally with the pars tuberalis, and ventrally with the bulk of the anterior lobe. Histologically it is characterized by a lack of eosinophile cells.



Text-fig. 1. Diagram of a sagittal section through the hypothalamus, pituitary and associated vessels of the rabbit. *A*--*A*, plane of the diaphragma sellae, which is pierced by the zona tuberalis, and more posteriorly by the infundibular stem; *A.H.* anterior hypophysial artery; *I.C.* internal carotid artery; *I.P.* infundibular process; *O.C.* optic chiasma; *P.D.* pars distalis; *P.H.* posterior hypophysial artery; *P.T.* pars tuberalis; *Z.T.* zona tuberalis.

During an experimental investigation (for another purpose) in which serial sections through the hypophysis and surrounding structures of many rabbits were studied, it was noted that the morphology of the zona tuberalis bore a close relationship to the vascular pattern of the region. In cross-section the zona tuberalis appeared to contain many large vascular sinusoids (Pl. 1, fig. 1), and to be separated from the rest of the pars distalis by a branch of the internal carotid artery running in connective tissue into the interior of the gland (Pl. 1, figs. 1, 2). The sections on which these observations were made had been

prepared after perfusing the blood vessels with saline and fixative, and were therefore unsatisfactory for the detailed study of the vessels.

The large sinusoids in the zona tuberalis have been investigated in collaboration with Dr J. D. Green (Green & Harris, 1947), and have been found to be the trunks of the hypophysial portal vessels. The portal vessels start as a multitude of capillary loops in the median eminence of the tuber cinereum. These unite to form large trunks, which pass through the zona tuberalis in a vertical plane, i.e. along the longitudinal axis of this cylindrical structure, eventually dividing into smaller branches which pass caudo-ventrally into the sinusoids of the pars distalis (Text-fig. 1).

The systemic arterial supply to the rabbit's hypophysis has not been clearly described. Wislocki & King (1936) mention two routes of arterial supply for the gland in rabbits, cats and monkeys. According to these authors the superior hypophysial arteries arise from the internal carotid or posterior communicating arteries, run down the pituitary stalk and supply the anterior lobe. The inferior hypophysial arteries arise from the internal carotids as they lie in the cavernous sinuses, run medially, unite in the mid-line and send branches into the posterior pole of the gland to supply the infundibular process and pars intermedia. Brooks & Gersh (1940) mention briefly various sets of arteries supplying the rabbit's hypophysis. Since our chance observations were not in agreement with these descriptions, and since a great deal of experimental work has been performed on the rabbit's gland, the systemic blood supply and drainage of this structure was reinvestigated.

MATERIAL AND METHODS

Thirty-two rabbits of varying ages, sex and reproductive state have been injected with india ink masses. In eight cases the vascular supply of the pituitary gland was dissected under a binocular microscope; in twenty cases a block of tissue containing the hypothalamus, pituitary and surrounding structures was decalcified and sectioned serially; in four cases the arterial supply was first dissected, the specimen photographed, and then sectioned serially. Various injection masses have been used: (*a*) india ink in distilled water, 1 : 3; (*b*) india ink in water (1 : 3) to which has been added rice starch (20 g./33 c.c.); (*c*) india ink in 30 % gelatin solution (1 : 3); (*d*) equal parts of india ink, 30 % gelatin and rice starch (20 g./33 c.c.). The addition of gelatin to the mass is advantageous when the specimen is to be dissected under water. The rice starch enables a clear picture of either the arterial or venous pattern to be obtained (depending on the vessel injected), for the rice granules are of sufficient size to prevent penetration of the injected material into the capillaries (Langley, 1925). The injection of the ink-gelatin-starch mass needs care. The ink and gelatin solutions may be mixed and kept warm in a water bath, but the starch should be added only immediately prior to injection, for the final solution coagulates within a few minutes and is then useless. 5–10 c.c. of this solution suffice to fill the cerebral arterial tree.

RESULTS

(*a*) *The systemic arterial supply*

The rabbit's hypophysis is supplied by two arteries which will be referred to in this paper as the anterior and posterior hypophysial arteries.

The anterior hypophysial artery is a branch of the internal carotid artery, taking origin from this vessel as it runs upwards on the medial side of the anterior clinoid process. Since the point of origin is below the plane of the diaphragma sellae, this vessel passes immediately into the fibrous tissue of the pituitary capsule and is carried into the interior of the gland with the connective tissue that separates the zona tuberalis from the rest of the gland. In most injected specimens in which the skull was opened, the brain removed and the pituitary exposed from above, this vessel was clearly seen (Pl. 1, figs. 5, 6; Pl. 2, fig. 7). It was found to be a unilateral structure, very commonly present on the left side. At its origin it may be seen shining through the diaphragma sellae (Pl. 1, fig. 5; Pl. 2, fig. 7), and then pursuing a tortuous course posterio-medially, it gradually disappears from view as it recedes from the surface. In serial sections the tortuous nature of this vessel is well shown (Pl. 1, figs. 2, 4), and it is seen to enter the gland between the zona tuberalis and the rest of the pars distalis. On nearing the midline the anterior hypophysial artery divides into large branches which penetrate posteriorly into the pars distalis, and may be clearly followed in the (ink-starch-gelatin) injected specimens (Pl. 1, fig. 4). Some of these branches cross the midline so that the distribution of this vessel is bilateral. The region supplied is the pars distalis (except the postero-superior corners).

The posterior hypophysial artery, also a unilateral structure, arises from an internal carotid artery in the region of the anterior clinoid process. Macroscopically the posterior hypophysial artery may be seen to run backwards on the diaphragma sellae, ventral to the adjacent oculomotor nerve (Pl. 1, fig. 5). In this part of its course it is irregularly tortuous and variably placed. On reaching the plane of the posterior lobe it passes medially across the upper surface of the pituitary (Pl. 1, figs. 5, 6), in a wide curve convex posteriorly. This vessel also has a bilateral distribution. It crosses the midline, and terminates usually by anastomosing with one of the meningeal arteries of the opposite side. The region supplied by this artery includes the posterior lobe (infundibular process and pars intermedia), and the postero-superior corners of the pars distalis. This latter fact may be clearly seen by reference to Pl. 1, fig. 6, showing the upper surface of a pituitary gland partially injected with the ink-gelatin mass. In serial sections the course of this vessel and its distribution were confirmed. Branches were seen passing from the artery, through the diaphragma sellae, downwards into the adjacent gland tissue (Pl. 2, fig. 8).

(*b*) *The systemic venous drainage*

The observations of Popa & Fielding (1930, 1933) in man, and of Wislocki & King (1936) in the monkey and cat, that the venous effluent from the pituitary

passes laterally into the adjacent venous sinuses, has been confirmed in the case of the rabbit (Pl. 2, fig. 10).

(c) *Further observations on the vessels of the median eminence of the tuber cinereum*

Green & Harris (1947) have described small arterial branches from the circle of Willis supplying the large vascular plexus situated between the pars tuberalis and the tuber cinereum. From this plexus, characteristic capillary loops and tufts were found to penetrate the median eminence of the tuber cinereum, and to drain via the portal trunks in the zona tuberalis. These points have been repeatedly confirmed for the rabbit in the present study, and further observations have been made.

On removing the brain from the skull the tuber cinereum is very frequently torn away from the hypothalamus, and remains attached to the posterior lobe by the pituitary stalk. If the infundibular recess of the third ventricle is examined in such a specimen, a speckled appearence of the ependyma may be observed, the black stipples representing the summits of the capillary loops which have reached a sub-ependymal position. If the brain is carefully removed and the pituitary stalk cut so that the tuber cinereum remains attached to the hypothalamus, inspection of the base of the brain reveals clearly the different vascular fields of the hypothalamus, and of the median eminence of the tuber cinereum. The portal vessels start as small tributaries which run towards the centre of the conical tuber, uniting into larger trunks on the way (Pl. 2, fig. 11). The large portal trunks pass on to the anterior surface of the neural stalk, and then ventrally into the zona tuberalis whilst the stalk or infundibular stem sweeps almost horizontally backwards. These observations may be confirmed by reference to Pl. 3, figs. 12–23, showing a series of horizontal sections through this region in a rabbit injected with india ink. The mass of capillary loops in the median eminence of the tuber cinereum is well shown in Pl. 3, figs. 12–17, and these vessels may be observed to unite into larger trunks (Pl. 3, figs. 17, 18), and to pass ventrally into the zona tuberalis anterior to the neural stalk (Pl. 3, figs. 18–20).

(d) *The zona tuberalis*

This division of the glandular lobe of the rabbit's pituitary consists of a cylindrical mass of cells arranged in a vertical plane, continuous dorsally with the pars tuberalis, and ventrally with the main part of the pars distalis. The zona contains the middle trunks of the portal vessels, which in turn are continuous dorsally with the mass of capillaries in the median eminence, and ventrally with the sinusoids of the pars distalis. These portal trunks, injected with an india ink mass, are easily seen if a transverse section of the zona tuberalis is examined under a binocular microscope. As described by Tilney (1914) the pars tuberalis encircles the tuber cinereum like a collar. If traced ventrally the tuber cinereum passes into the neural stalk which sweeps posteriorly and downwards, whilst the pars tuberalis, passing on to the

anterior surface of the neural stalk, is continuous with the zona tuberalis which lies in a more vertical plane (Text-fig. 1). Thus the two structures diverge as they pass downwards and, if viewed from above, the zona tuberalis may be seen to pierce the diaphragma sellae by a large foramen situated anterior to that transmitting the neural stalk (Pl. 1, fig. 6). The postero-lateral borders of this foramen usually have a silvery appearance due to the thick connective tissue trabeculae which penetrate the gland here, and partially separate the zona tuberalis from the rest of the pars distalis. It is here (usually on the left side) that the anterior hypophysial artery enters the gland (Pl. 1, fig. 6).

In a small number of rabbits the large zona tuberalis appeared to be interrupted at the diaphragma sellae, and in these cases the portal vessels and the pars tuberalis passed through the foramen with the neural stalk, as is usually the case in other mammals.

DISCUSSION

The systemic arterial supply of the hypophysis is a consideration of some importance in various experimental procedures relating to this gland. In investigations dealing with the nerve supply of the pituitary gland in rabbits, the pituitary stalk has been sectioned to study the effect of this procedure on the reproductive processes. Harris (1937), Hinsey (1937), and Westman & Jacobsohn (1937) described a consequent genital atrophy, whilst Brooks (1938) obtained rabbits with genitalia characteristic of oestrus (apart from the fact that ovulation did not follow coitus). One possible explanation of this difference is that the first group of workers had incidentally damaged the arterial supply of the gland when cutting the stalk. The observations described in this paper make such an accident seem improbable. The major artery to the anterior lobe of the pituitary of the rabbit, the anterior hypophysial artery, arises below the level of the diphragma sellae, which would obviate any risk of trauma to this vessel on a frontal or temporal approach to the hypophysial stalk. The more likely explanation of the above discrepancy may be found in varying degrees of regeneration of the hypophysial portal vessels which were cut with the sectioned stalk. Brooks (1938) certainly states that in his rabbits these vessels were found, post-mortem, either to have been incompletely cut at operation or to have undergone regeneration.

Dawson (1937) suggested that the zona tuberalis is derived embryologically from the lateral lobes of Rathke's pouch. Atwell (1918*a*) gave a detailed description of the development of the pituitary gland of the rabbit, and described the formation of the pars tuberalis from bilateral evaginations from Rathke's pouch, the lateral lobes, which grow dorsally to the tuber cinereum and extend around the tuber anteriorly and posteriorly to form a collar-like structure. The final 'stalk' of these lateral lobes, according to Atwell, largely disappears, remaining only as a small cortical zone at the anterior pole of the gland. Evidence for Dawson's view that these lateral lobes besides forming the pars tuberalis form the zona tuberalis also, has been found in the glands of two rabbits in which the zona tuberalis was seen to be a bilateral structure (Pl. 2,

fig. 9). This may represent incomplete fusion of the lateral lobes. If Dawson's view of the origin of the zona tuberalis is correct, it would seem likely that this structure may be represented by some part of the hypophysis in other forms for the development of the pars tuberalis from lateral lobes in many animals has been described (Atwell, 1918*a* (rabbit), 1918*b*, 1921 (amphibians), 1926 (man); Atwell & Sitler, 1918 (chick); Baumgartner, 1916 (reptiles); Tilney, 1914 (cat and fowl); Parker, 1917 (marsupials); Wheeler, 1943 (opossum); Schwind, 1928 (rat); Nelson, 1933 (pig); House, 1943 (ox); Brahms, 1932 (cat)).

The gross structure of the zona tuberalis depends on several factors such as (*a*) the length and direction of the pituitary stalk, and (*b*) the vascular pattern of the region. In man the stalk is relatively long and slopes downwards and forwards, instead of downwards and backwards as in the lower mammals. It might be expected, then, that any representative of the zona tuberalis in man would be a stretched, attenuated structure. In human material, as described by Green & Harris (1947), the portal vessels may be observed to sweep to the anterior surface of the pituitary stalk, and thus into the pars distalis. Possibly this junctional region between stalk and pars distalis, which contains the trunks of the portal vessels, is representative of the zona in man. Atwell (1926) has suggested that this region may be of importance in the development of Erdheim's tumours.

Since the method of development of the pars tuberalis from the lateral lobes of Rathke's pouch is constant over a wide variety of vertebrates, the question arises as to the functional significance of the definitive structure (pars tuberalis $\pm$ zona tuberalis). In view of the fact that the pars tuberalis has no proven endocrine function, the suggestion is put forward that this part of the pituitary is established to transmit an anatomical (vascular) pathway from the neurohypophysis to the pars distalis. This vascular link has now been found in the rat, guinea-pig, rabbit, cat, dog, monkey and man. Some mammals, such as the elephant (Wislocki, 1939), whale (Wislocki & Geiling, 1936), sea-cow (Oldham, McCleery & Geiling, 1938) and porpoise (Geiling, Voss & Oldham, 1940), possess a connective tissue septum separating the infundibular process from the pars distalis; but it is known that the pars tuberalis still extends upwards to the tuber cinereum in front of this septum. It has been shown (Harris, 1947) by india ink injection that in the porpoise the pars tuberalis and what appears to be a zona tuberalis transmit a well-marked hypophysial portal system of blood vessels from the median eminence to the pars distalis. Thus it would seem that in a wide variety of animals the mode of development of the pars tuberalis is constant, the attachment of the pars tuberalis to the tuber cinereum is constant, and that so far as observations have been made, the pars tuberalis bears a constant relationship to the hypophysial portal system. Harris (1944), and Green & Harris (1947), have summarized the evidence that hypothalamic control over the activity of the pars distalis is transmitted by these vessels.

SUMMARY

1. The vascular system of the pituitary gland of the rabbit has been studied, following injection of various india ink masses.

2. The systemic arterial supply of the gland consists of two asymmetrical branches from the internal carotid arteries. The anterior hypophysial artery arises usually from the left internal carotid below the plane of the diaphragma sellae, and enters the gland with connective tissue between the zona tuberalis and the remainder of the pars distalis. The posterior hypophysial artery arises from the left or right internal carotid artery, courses posteriorly and then sweeps across the upper surface of the diaphragma sellae. The distribution of both vessels is bilateral, the anterior hypophysial artery supplying the greater part of the pars distalis, and the posterior supplying the infundibular process, pars intermedia, and postero-superior corners of the pars distalis.

3. The systemic venous drainage passes laterally into the adjacent venous sinuses.

4. A previous description of the hypophysial portal circulation has been confirmed, and extended by observations obtained by dissection of the vessels under the binocular microscope.

5. The zona tuberalis is occasionally bilateral. This observation supports the view that it develops from the lateral lobes of Rathke's pouch.

6. The functional significance of the pars and zona tuberalis is discussed.

It is a pleasure to record my thanks to Prof. H. A. Harris for his ever-willing advice and encouragement. I am also grateful to Mr J. A. F. Fozzard for his skilful photography, and to Mr R. Smith for his valuable aid in the preparation of the histological material.

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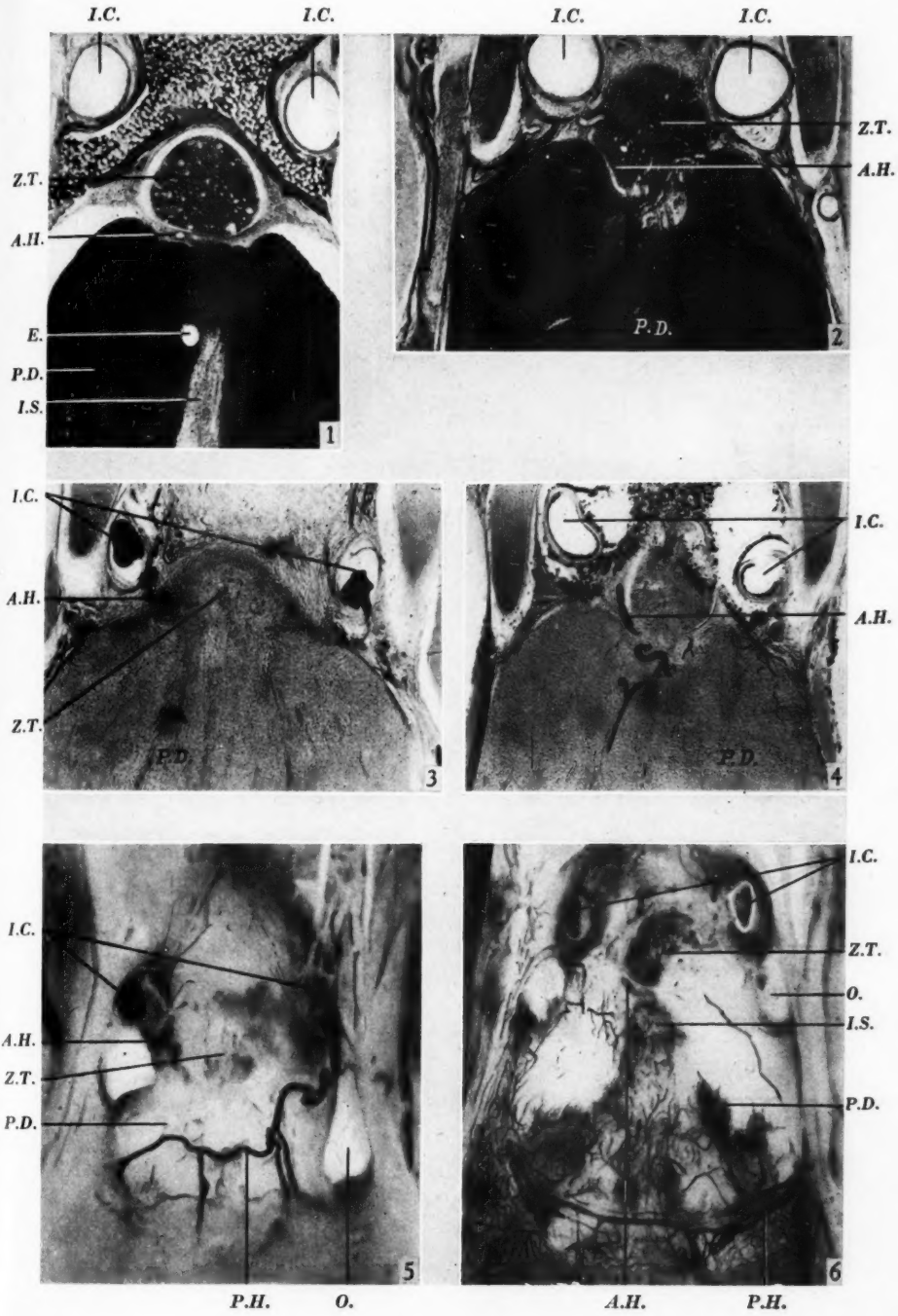
EXPLANATION OF PLATES

Figs. 1–23 are unretouched photographs of the pituitary gland and associated structures of the rabbit. All the sections are 100μ thick.

The following abbreviations are used: *A.H.* anterior hypophysial artery; *I.C.* internal carotid arteries; *I.P.* infundibular process; *I.S.* infundibular stem; *O.* oculomotor nerve; *O.C.* optic chiasma; *P.D.* pars distalis; *P.H.* posterior hypophysial artery; *P.I.* pars intermedia; *T.C.* tuber cinereum; *Z.T.* zona tuberalis.

Plate 1

Fig. 1. Horizontal section through pituitary gland of animal used in experimental investigation. Fixation effected by perfusing the vessels with formalin. Note the large dilated sinusoids (empty) in the zona tuberalis, and the anterior hypophysial artery (*A.H.*) cut transversely in the connective tissue between the zona tuberalis and the rest of the pars distalis. *E.*—hole left by electrode after fixation *in situ*. ×16·8.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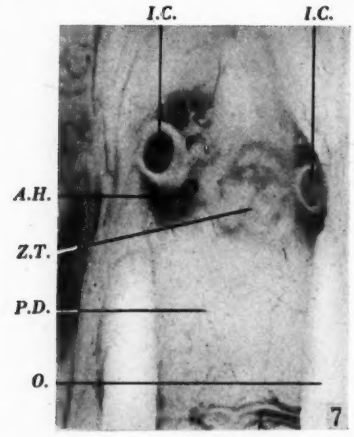
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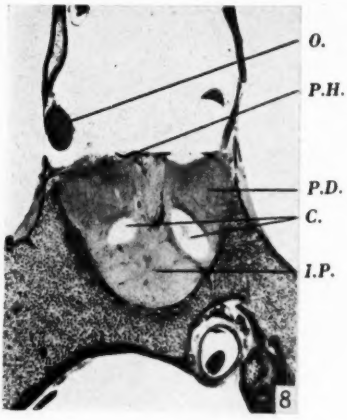
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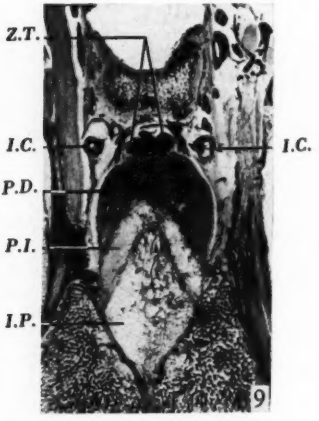
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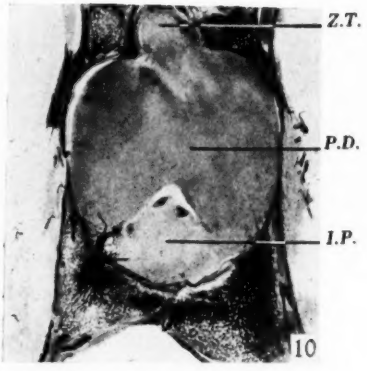
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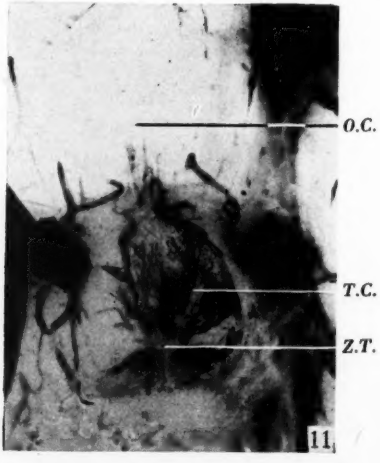
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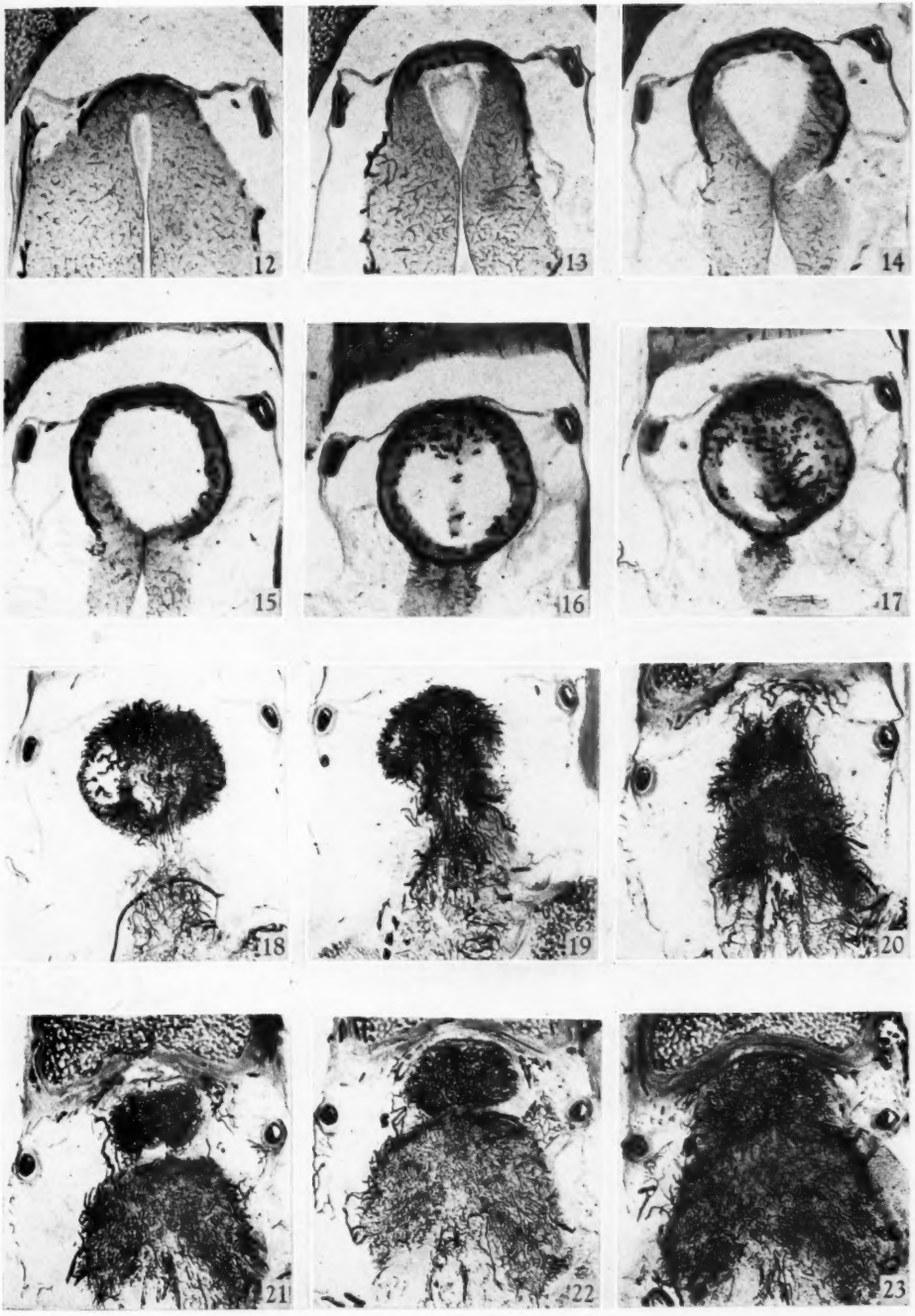
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Fig. 2. Horizontal section through pituitary gland of animal used in experimental investigation. Fixation effected by perfusing the vessels with formalin. Note the anterior hypophysial artery (*A.H.*) arising from the left internal carotid artery (*I.C.*) and entering the antero-lateral aspect of the gland. × 16·8.

Fig. 3. Oblique section through the pituitary gland shown in Pl. 1, fig. 5. Arteries injected with the ink-gelatin-starch mass. Note the anterior hypophysial artery (*A.H.*) arising from the left internal carotid artery and entering the antero-lateral aspect of the gland. × 16·8.

Fig. 4. Horizontal section through the pituitary gland shown in Pl. 2, fig. 7. Arteries injected with ink-gelatin-starch mass. Note the anterior hypophysial artery (*A.H.*), arising from the left internal carotid artery and entering the antero-lateral aspect of the gland between the zona tuberalis and the rest of the pars distalis. × 16·8.

Fig. 5. Photograph of the superior surface of the pituitary gland *in situ*. Same specimen as that shown in Pl. 1, fig. 3 and Pl. 2, fig. 8. Arteries injected with the ink-gelatin-starch mass. Note the anterior (*A.H.*) and posterior (*P.H.*) hypophysial arteries. × 10.

Fig. 6. Photograph of the superior surface of pituitary gland *in situ*. Vessels injected with india ink solution. Note the anterior (*A.H.*) and posterior (*P.H.*) hypophysial arteries. The incomplete filling of the anterior vessel demonstrates the area of supply of the posterior hypophysial artery. × 10.

PLATE 2

Fig. 7. Photograph of the superior surface of pituitary gland *in situ*. Same specimen as that shown in Pl. 1, fig. 4. Arteries injected with ink-gelatin-starch mass. Note the anterior (*A.H.*) and posterior (*P.H.*) hypophysial arteries. × 10.

Fig. 8. Oblique section through the pituitary gland shown in Pl. 1, fig. 5. Arteries injected with ink-gelatin-starch mass. Note the posterior hypophysial artery (*P.H.*) passing transversely across the upper surface of the diaphragma sellae. *C.* cysts in the pars intermedia. × 8.

Fig. 9. Horizontal section through the pituitary gland. Vessels injected with ink-gelatin mass. Note the bilateral zona tuberalis (*Z.T.*). × 8.

Fig. 10. Horizontal section through the pituitary gland. Veins injected with ink-gelatin-starch mass. Note the large vein draining from the postero-lateral aspect of the gland into the adjacent venous sinuses. × 8.

Fig. 11. Photograph of base of the brain. Vessels injected with ink-gelatin mass. Brain from the same animal as that from which the pituitary gland shown in Pl. 1, fig. 6 was obtained. Note the distinct vascular field of the tuber cinereum. This specimen shows the commencement of the hypophysial portal vessels as they collect into large trunks and converge towards the zona tuberalis (*Z.T.*). × 10.

PLATE 3

Figs. 12–23. Serial sections (horizontal plane) through the tuber cinereum and pituitary gland to show the vascular relations of the zona tuberalis. Vessels injected with india ink. (Fig. 12, section 2; fig. 13, section 4; fig. 14, section 6; fig. 15, section 7; fig. 16, section 8; fig. 17, section 9; fig. 18, section 10; fig. 19, section 11; fig. 20, section 12; fig. 21, section 13; fig. 22, section 14; fig. 23, section 15.) Note the pars tuberalis, with its associated vascular plexus, on the anterior surface of the tuber cinereum (fig. 12), and at lower levels encircling the tuber cinereum (fig. 16). Coextensive with the pars tuberalis are the sinusoidal loops which penetrate the median eminence of the tuber cinereum (figs. 12–17). The vessels unite into large trunks (figs. 17, 18), which pass ventrally in the zona tuberalis (figs. 18–22), and finally merge with the sinusoids of the pars distalis (fig. 23). The anterior hypophysial artery may be seen entering the gland with the connective tissue which separates the zona tuberalis from the more posterior part of the pars distalis (fig. 22). Magnification of figs. 12–23, × 16.

THE DISTRIBUTION OF ALKALINE PHOSPHATASE IN THE PREGNANT UTERUS OF THE RAT

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INTRODUCTION

The pregnant uterus and foetal membranes are known both on chemical (Busse, 1936) and histochemical (Dempsey & Wislocki, 1946) grounds to contain large quantities of alkaline phosphatase, but apart from Hard's (1946) work on the guinea-pig, the distribution of this enzyme in any one species has not been studied from the beginning to the end of pregnancy. Wislocki & Dempsey (1945, 1946*a*, *b*) and Wislocki, Deane & Dempsey (1946) in the course of a large-scale histochemical survey of the pregnant uterus have established the fact that there are major species differences in the distribution of the enzyme. Their work, therefore, emphasizes the need for each species to be studied separately and in detail.

The fundamental significance of alkaline phosphatase during pregnancy is not known with any certainty, but one reasonable hypothesis is that it is concerned in the transference of materials to and from the foetus. Data about the distribution of the enzyme in organs such as the decidua, yolk-sac and allantoic placenta, which play major roles in foetal nutrition, should therefore be of value in deciding for or against this hypothesis.

It was therefore decided to make a detailed histochemical investigation of the uterus of the rat for alkaline phosphatase throughout pregnancy.

MATERIAL AND METHODS

The rats used in this investigation were bred from albino and black strains of *Mus norvegicus*. The date of copulation was determined from daily vaginal smears, and the day after copulation was counted as the first day of pregnancy. The rats were killed at oestrus and at daily intervals thereafter until the end of pregnancy. The uterus and a piece of small intestine were removed at the same time and fixed in absolute alcohol, the latter organ serving as a control. The larger gestation swellings were slit anti-mesometrially to facilitate penetration by the fixative.

Sections were cut in paraffin in the usual way, a standard thickness of 8 μ being maintained throughout the investigation. Sections were floated on 90 % alcohol before mounting. After removal of the wax the sections were coated with 0·5 % celloidin in alcohol-ether before proceeding to water. They were incubated for 2 hr. at 37° C., with periodic agitation, in a substrate mixture

composed of: 2% sodium glycerophosphate, 1 part; 2% calcium nitrate, 1 part; distilled water, 6 parts; and sufficient 0·1% sodium hydroxide to adjust the pH of the solution to 9·5. The precipitate of calcium phosphate at sites of alkaline phosphatase activity was then made visible by converting it to cobalt sulphide. It will be realized that this procedure is essentially that of Gomori's (1939) and Takamatsu's (1939) original techniques. Results were not so good, however, when floating on alcohol and coating with celloidin were omitted. The addition of M/1000-magnesium chloride to the substrate mixture did not materially affect the results.

On examination of the stained sections, sites of enzyme activity appear as light brown to black deposits of cobalt sulphide. Provided the cell is not overstained, the deposit can be located with great accuracy in such parts as the nuclear membrane, nucleoli and peripheral cytoplasm, and the colour of the deposit gives an indication of the degree of activity at each site. A black deposit tending to obliterate cell details because of its density was regarded as indicative of intense enzymic activity; a dark brown deposit, of moderate activity; light brown, of slight activity; and just perceptible staining, of minimal activity. It was not justifiable to carry quantitative analyses further than this. Throughout this paper the terms 'intense', 'moderate', 'slight' and 'minimal' will be used in these restricted senses, though occasionally 'heavy' may be substituted for 'intense' and 'light' for 'slight' where the words qualify 'staining' rather than 'activity', although these two latter terms will be regarded as equivalent and interchangeable.

Of course, if incubation is carried on long enough, previously moderate staining gives place to intense staining, and so on. It was found by experience, however, that maximum separation, or contrast, was obtained with 8 μ sections after 2 hr. incubation at 37° C., and at a pH of 9·5, provided that the substrate was agitated at 15 min. intervals.

The terminology employed for the decidua and foetal membranes is due to Krehbiel (1937) and Mossman (1937) respectively.

RESULTS

The alkaline phosphatase activity of the pregnant uterus showed three well-defined phases: (1) before implantation (5th day) overall activity was slight, although the surface and glandular epithelium, leucocytes and vascular endothelium (Pl. 1, fig. 1) were heavily stained; (2) from implantation until the foetal vascularization of the allantoic placenta at the 11th day, the decidua showed intense activity (Pl. 1, figs. 3–5); (3) from the 11th day until full-term, activity was chiefly confined to the foetal membranes (Pl. 2, figs. 6–9).

Compared with that of the decidua and foetal membranes, the enzyme activity of the embryo and foetus was slight until the last week of pregnancy, when the developing bones and some other organs exhibited intense activity (Pl. 2, fig. 9).

Within the periods mentioned the overall distribution of activity did not vary greatly. Individual tissues, however, showed progressive changes in the details of their enzyme distribution. The results are therefore best considered tissue by tissue rather than in strict chronological order.

MATERNAL TISSUES

Surface epithelium of the uterus

From the time of copulation until the 4th day the nuclei showed slight activity, while the cytoplasm adjacent to the lumen showed a band of intense activity (Pl. 1, fig. 2). Within the nucleus the nuclear membrane, nucleoli, chromosomes and chromatin granules were most heavily stained, so that appearances were very similar to those of cells stained with haematoxylin. At the 5th day the enzyme disappeared from the epithelium, not only in the implantation crypts, but also generally throughout the uterus. At the 13th day the enzyme reappeared and was thereafter to be found at similar sites to those just described until full term. It is to be noted that this waning and waxing of enzyme activity paralleled the decreasing and increasing of the height of the epithelial cells which Selye, Collip & Thomson (1935) have described as part of their 1st and 2nd progestational responses, and which are attributable to changes in the oestrogen/progesterone ratio in the circulating blood.

Glandular epithelium

The distribution of the enzyme of this epithelium was similar to that of the surface epithelium. The enzyme, however, did not disappear from the former until the 6th day and did not reappear until the 14th day. The enzyme was also present in the lumina of some of the glands, which suggested that it might be a secretory product. On the other hand, it may have been carried there by leucocytes which had passed through the epithelium, for degenerating polymorphs were frequently to be seen within the ducts.

Uterine stroma

The nuclei of the stroma cells showed slight activity during the first 3 days of pregnancy. On the 4th day they became more heavily stained in the immediate neighbourhood of the glands and surface epithelium. With the onset of the decidual reaction on the 5th day the staining of the nuclei and later of the cytoplasm became intense (Pl. 1, fig. 3; Pl. 3, figs, 10, 11 and 13). Between the decidual swellings, however, the stroma remained in the same state as regards phosphatase activity as that found during the first 4 days.

Muscularis

Staining of the plain muscle of the uterine wall was minimal at all stages of pregnancy.

Vascular endothelium

Until the 5th day the nuclei and cytoplasm of the vascular endothelium in all parts of the uterus stained intensely. The vessels of the muscularis and non-decidual stroma continued to show activity throughout pregnancy, but those of the decidua lost their activity, except mesometrially, where the endothelium continued to stain in a narrow zone adjacent to the muscularis (Pl. 2, fig. 6). At the time of formation of the metrial gland in mid-pregnancy the vessels of the mesometrium also lost their activity.

Leucocytes

During the first 3 days after copulation the stroma showed a relatively heavy infiltration with polymorphonuclear leucocytes. Similar cells were present between the epithelial cells and within the lumen. They were characterized by intense nuclear phosphatase activity. In the middle stages of pregnancy the maternal sinuses of the decidua basalis also showed a moderate number of these heavily stained cells.

Decidua

The decidual reaction appeared on the 5th day and was characterized initially by intense staining of all its zones. At the periphery of each decidual swelling the staining passed off abruptly into that of the normal stroma.

Staining, however, did not remain uniform throughout the decidua (Pl. 1, figs. 3–5). Soon after the first appearance of the reaction a number of well-defined zones could be differentiated on the basis of differences in staining intensity and in the proportionate activity of nuclei and cytoplasm. By the 8th day (Pl. 1, fig. 4) these differences had become very well marked indeed. Apart from these criteria the zones were also distinguishable by differences in the packing of the cells, the size of the cells and the arrangement of their blood vessels. The zones, in fact, were almost identical with those described by Krehbiel (1937) on other morphological and histochemical grounds. They were as follows (Pl. 1, fig. 4): in sections cut transversely across the uterus, which included the blastocyst in its crypt, there were (1) a mesometrial subepithelial zone of intense activity, (2) an anti-mesometrial subepithelial zone of relatively light staining, (3) a horseshoe-shaped anti-mesometrial zone of intense activity, (4) wing-like lateral mesometrial zones of relatively slight activity, (5) a central mesometrial zone of intense staining and (6) a marginal zone, adjacent to the circular muscle, of extremely heavy staining. With later modifications in the decidua, zone (3) became the definitive decidua capsularis, while zones (4) and (5) combined to form the decidua basalis (Pl. 1, fig. 5).

On the 10th day enzyme activity was still intense in most parts of the decidua, the lightest staining areas being the glycogenic zones of the basalis. On the 11th day the decidua normally begins to atrophy, and it was observed that at this time the phosphatase activity was rapidly lost from all regions

except the central mesometrial zone and the more peripheral parts of the basalis where the endothelium of the maternal blood vessels stained intensely. By the 12th day the decidua had lost all its activity.

It is important to note that those decidual zones (viz. (2) and (4)) which from the beginning showed least phosphatase activity were precisely those which Krehbiel (1937) showed to accumulate glycogen, while the decidua capsularis, which stained very heavily for phosphatase, stored lipoids but was wholly devoid of glycogen.

Another feature of note was that when phosphatase activity became pronounced in the decidual cells on the 5th day, it was confined to the nuclei (Pl. 3, fig. 10). On the 6th day the cytoplasm began to stain in a diffuse manner, but less heavily than the nuclei (Pl. 3, fig. 11). On the 7th day conditions were reversed and cytoplasmic staining was heavier than nuclear; moreover, the enzyme now became concentrated at the surface of the cells. On the 8th day this change had progressed to the point where all cytoplasmic activity was peripheral and the nuclei stained but slightly (Pl. 3, fig. 13). Not all aspects of the cell surface showed a similar degree of enzyme concentration, however; in many places it was most marked on the side facing a blood vessel, but this relationship was by no means absolute.

Metrial gland

Between the circular and longitudinal muscle layers at the attachment of the mesometrium the perivascular accumulations of binucleated cells which constitute the metrial gland of Selye & McKeown (1935) did not show phosphatase activity. The normal vascular endothelium here likewise showed no activity. However, coursing in a spiral manner through the centre of the gland, there were normally one or two wide vessels lined irregularly by three to four layers of very large cells. These cells showed intense activity by contrast with the endothelium of the normal vessels which showed none (Pl. 3, fig. 15). In serial sections cut tangentially these specialized blood vessels could be traced through the centre of the decidua basalis into the spongy zone of the foetal placenta where their lining cells were continuous with the trophoblast. The large cells mentioned have usually been regarded as hypertrophic maternal endothelial cells (Mossman, 1937), but from the evidence given it seems more likely that they are of trophoblastic origin, especially since they stain for phosphatase with similar intensity to that of the trophoblastic cells of the spongy zone (Pl. 2, fig. 6).

FOETAL TISSUES

Blastocyst

Until the 9th day the blastocyst showed little phosphatase activity (Pl. 1, fig. 3). After prolonged incubation (12 hr.) most nuclei showed slight activity, but after the standard 2 hr. incubation, staining was minimal. On the 9th day the ectodermal cells, both at the surface and within the blastocyst, began to show increased activity. The entoderm remained unstained.

The heaviest staining occurred in the mononuclear giant cells lying adjacent to the decidua. Here not only the nuclei but also the thin cytoplasmic processes of the cells were heavily stained. In view of later findings it is important to note that this first indication of cytoplasmic activity in the trophoblast occurred in cells which are bathed by maternal blood, and which form a part of the yolk-sac placenta whose functional importance at this stage of pregnancy has been clearly demonstrated (Everett, 1935).

At the 10th day (Pl. 1, fig. 5) the moderate to intense staining of the trophoblast was in marked contrast with the minimal staining of the embryo (excepting the neural tube), yolk-sac entoderm and allantoic mesoderm. The mononuclear giant cells showed intense nuclear and peripheral cytoplasmic activity. The spongy zone showed intense nuclear activity throughout, but intense cytoplasmic activity only at the surface of cells bathed directly by the maternal blood stream. The nuclei of the chorionic-plate trophoblast were also intensely active at this stage.

Allantoic placenta

On the 11th day (Pl. 2, fig. 6) the allantoic mesenchyme and blood vessels had begun to invade the chorionic plate. This mesenchyme differed from that of the allantoic stalk in that the former showed moderate nuclear activity while the latter was unstained. The distribution of activity in the trophoblast was similar to that of the previous day, the most marked feature again being the intense cytoplasmic activity of those parts of the trophoblast bathed with maternal blood.

Labyrinth

Between the 12th and 16th day the labyrinth consisted partly of syncytial trophoblast lining maternal blood channels, and partly of islands of cytotrophoblast (Pl. 4, fig. 16). The former continued to show intense cytoplasmic staining, while the latter gradually lost its nuclear activity. At the 16th day every maternal blood channel was clearly defined by lines of intense staining against an unstained background (Pl. 4, fig. 17). After the 16th day the staining of the syncytium became less regular but no less intense. At full term, under high magnification, it was seen to have lost its evenness and to consist of discrete clumps of precipitate of varying size which were chiefly concentrated around the nuclei. Between the nuclei, however, there was sufficient stain to make it possible to assert that there were no gaps in the syncytium. In the light of this observation it is difficult to agree with Mossman (1937) that a haemo-endothelial relationship exists in late pregnancy in the rat. On similar grounds Hard (1946) was unable to accept the view that the guinea-pig's placenta was haemo-endothelial.

Spongy zone

As in the labyrinth, the maternal blood channels of the spongy zone remained clearly outlined throughout pregnancy (Pl. 2, fig. 8 and Pl. 4, fig. 18). Unlike the labyrinth, however, some activity persisted in the nuclei until full

term. The binucleated glycogenic cells which normally accumulate in immense numbers in this zone between the 14th and 18th days never showed more than minimal activity. On morphological and histochemical grounds, therefore, these cells were identical with those of the metrial gland, an observation which supports Szendi's (1933) and Selye & McKeown's (1935) claims that all glycogenic cells have a common origin in the adventitia of the mesometrial blood vessels, and reach the spongy zone by migration.

Giant cell zone

Throughout pregnancy these cells showed strong cytoplasmic and moderate nuclear activity.

Allantoic mesoderm

After their initial invasion of the labyrinth on the 11th day, the foetal connective tissue and vascular endothelium rapidly lost their phosphatase activity. From the 14th day the labyrinth showed clear areas occupied by foetal connective tissue alternating in a striking manner with heavily outlined maternal channels (Pl. 4, fig. 17).

Entodermal sinuses

Beginning at the 14th day the entering and emerging allantoic vessels and their connective tissue coverings acquired double-walled omphalopleuric sheaths. Reichert's membrane and the entodermal cells comprising these sheaths showed slight activity until full term.

Yolk-sac

(1) *Omphalopleure.* The giant cells showed intense, but the entodermal cells only slight, activity.

(2) *Splanchnopleure.* Until the 13th day the entodermal cells of the inner wall of the yolk-sac did not stain, but on that day their nuclei began to stain strongly (Pl. 3, fig. 12). This change coincided with the normal hyperplasia of the entoderm which results in the formation of villous projections over the mesometrial half of this part of the yolk-sac. On the 14th day the distal cytoplasm of the entodermal cells began to stain intensely, and this continued to be the case (Pl. 3, fig. 14) until the 19th day, when staining became less intense and more diffuse. Nuclear staining became less intense after its initial appearance on the 13th day (cf. Pl. 3, figs. 12, 14).

Amnion

During the latter half of pregnancy the amnion stained in an irregular manner. Sections gave the impression that the enzyme had been absorbed at the surface of the membrane rather than of having been manufactured locally.

FOETUS

The location of phosphatase activity in the embryo and foetus throughout pregnancy was not subjected to detailed investigation. Only a few points can be made here.

On the 10th day the nuclei of the neural tube showed moderate activity. On the 12th day the anterior horn cells and their axons were conspicuously stained compared with the rest of the nervous system. The Wolffian duct and the entoderm of the alimentary canal also showed some activity. On the whole, however, the tissues of the foetus proper were poorly stained as compared with the foetal membranes.

On the 14th day the condensed mesenchyme in the position of the future mandible and maxilla showed intense nuclear activity. When ossification commenced at these sites on the 16th day both the nuclei and the cytoplasm of the osteoblasts stained with great intensity, and the enzyme was also present intercellularly. At this stage hypertrophic cartilage also showed great activity. There was little activity in the remainder of the embryo. On the 19th day (Pl. 2, fig. 9), in addition to centres of ossification, organs such as the kidney, lung, salivary glands and pancreas, in which rapid proliferation of ducts was taking place, showed intense activity (Pl. 4, fig. 19); developing hair and teeth and the spinal ganglia were also active. In all these instances staining was predominantly nuclear and rivalled in intensity that of the foetal membranes. The activity of the liver was patchy, being heavy in areas showing active haematopoiesis, but light elsewhere.

DISCUSSION

The only other observations on the distribution of alkaline phosphatase in the decidua and foetal membranes of the rat which have been reported are those contained in papers by Wislocki & Dempsey (1945) and Wislocki *et al.* (1946), and with their findings, so far as they go, the observations made in this paper are in substantial agreement, although it has not been possible to subscribe fully to all their interpretations, particularly those concerning the role of the enzyme in glycogen synthesis. This point will be discussed later. The data given by Hard (1946) for the guinea-pig and by Wislocki & Dempsey (1945, 1946*a*, *b*), Wislocki *et al.* (1946) and Dempsey & Wislocki (1945, 1947) for man, the mouse, rabbit, guinea-pig, hamster, cat and sow, make it clear that the distribution of the enzyme varies greatly from species to species. The only constant finding was the presence of the enzyme in substantial amounts in either the trophoblast or the maternal endothelium between the foetal and maternal circulations. From this it might be inferred that alkaline phosphatase is intimately concerned in the transport of materials to and from the foetus. The enzyme, however, is not confined to sites where such exchange is taking place, so that it is necessary to consider other explanations of its presence.

Moog (1944) and Brachet (1946) found that alkaline phosphatase was most active at two stages in the life history of cells: (1) during proliferation and differentiation and (2) during specific functional activity. The observations reported here are in agreement with this view, but, in addition, they bring out the fact that during the former stage the enzyme was mainly active within the nucleus, while in the latter stage the activity was almost exclusively

cytoplasmic. Thus, in the decidua, trophoblast, inner wall of the yolk-sac and in the skeletal, glandular and nervous tissues of the foetus, the stage of rapid cellular multiplication and differentiation was associated with intense nuclear activity. On the other hand, the mature decidua, and those parts of the trophoblast and yolk-sac engaged in the transference of materials to and from the foetus, were characterized by intense activity of the peripheral cytoplasm. Where functional activity followed rapidly upon cellular proliferation, an intermediate stage was observed, in which heavy nuclear staining was combined with moderate, diffuse cytoplasmic staining. It would appear from this that the significances of nuclear and cytoplasmic phosphatase should be separately evaluated.

(1) The role of the nuclear phosphatases appears to be bound up with the metabolism of nucleoproteins, which in turn are intimately concerned in protein synthesis and cell division (Brachet, 1946). Thus the enzyme which breaks down nucleotides to nucleosides is an alkaline phosphatase (Sumner & Somers, 1947). It has been shown that the intensity of alkaline phosphatase activity runs parallel with the rate of synthesis of desoxynucleic acid (Brachet, 1946). A similar enzyme is present in large amounts in the chromosomes where its distribution corresponds closely with that of the Feulgen-staining desoxynucleic acids (Willmer, 1942; Krugelis, 1942; Danielli & Catcheside, 1945). In the developing chick Moog (1943) found a general correspondence between alkaline phosphatase activity and the rapidity of cell division, though in some cases the activity was better correlated with the stage of cellular differentiation than with mitosis.

Summing up we can say that the intensity of nuclear phosphatase staining is a useful index of the morphogenetic activity of the cell by virtue of the participation of the enzyme in the manufacture and degradation of nucleoproteins.

(2) The functions accorded to cytoplasmic phosphatases are numerous and varied, and in many cases appear to be specific for the cells concerned.

In certain glandular and other cells fine granules can be centrifuged off which are very rich in both ribosenucleic acids and alkaline phosphatase (Brachet & Jeener, 1944). In regenerating nerves the resynthesis of the nucleoprotein-containing Nissl substance is accompanied by intense acid phosphatase activity of the cell body (Bodian & Mellors, 1945). The phosphatases are not confined to the formed nucleoprotein elements of the cytoplasm, however, for they are also found free in the general cytoplasm (Brachet & Jeener, 1944). Recently, Jeener (1947) has suggested that the alkaline phosphatase of the cytoplasm is more particularly concerned in the manufacture of fibrous proteins such as keratin, collagen and myosin, than with general protein formation.

In ossifying and calcifying tissues an alkaline phosphatase has been shown to be responsible for the provision of inorganic phosphate ions from an unknown precursor (Robison & Soames, 1924). The enzyme is nuclear, cytoplasmic and intercellular in its distribution (Bourne, 1943).

The phosphatase of the liver appears to be responsible for the final stage in the breakdown of glycogen to glucose (Cori, Cori & Schmidt, 1939). On the other hand, in developing hair (Johnson & Bevelander, 1946), teeth (Horowitz, 1942; Bevelander & Johnson, 1945) and in the placenta (Wislocki *et al.*, 1946), alkaline phosphatase stains most heavily in regions which are apparently actively storing glycogen. Wislocki *et al.* (1946) therefore suggest that the enzyme takes part in glycogen synthesis. This view, however, is improbable on thermodynamic grounds (Soskin & Levine, 1946), and in any case glycogen synthesis in both liver and muscle apparently does not involve either acid or alkaline phosphatases (Barron, 1943). In this connexion it is of interest that in the present investigation the glycogen-storage zones of the decidua showed much less alkaline phosphatase activity than the fat-storage zones, while in the metrial gland, where glycogenesis is very active, the enzyme was restricted to the lining of the specialized blood vessels.

In the intestinal epithelium the alkaline phosphatases of the brush border and Golgi zones are probably concerned in the absorption of sugars (Lundsgaard, 1933) and fatty acids (Jeker, 1936). In the proximal convoluted tubules of the kidney also there is evidence that the phosphatase of the brush border is concerned in the absorption of glucose from the glomerular filtrate (Lundsgaard, 1933).

In the placental trophoblast and yolk-sac epithelium, the alkaline phosphatase is strategically placed for the performance of similar functions to those ascribed to the intestinal and renal-tubular phosphatases. The enzyme may therefore play a vital role in the transference of sugars and fatty acids. It must not be forgotten, however, that the demands of the embryo and foetus for inorganic phosphate are very great and that therefore the enzyme may be primarily concerned with the liberation of this substance from organic precursors in the maternal blood stream. The fact that the level of inorganic phosphate is higher in the foetal than in the maternal blood, while the reverse is true in the case of organic phosphate compounds, is in agreement with this point of view (Timpe, 1931).

Finally, however, it must be stressed that alkaline phosphatase almost certainly does not act alone, but that in all probability it merely forms one of a battery of enzymes concerned in the transference and metabolism of nutritive substances. Until more information is available about the other members of the system it is premature to speculate too far as to the functional importance of the one or two components which have been intensively studied.

SUMMARY

1. The distribution of alkaline phosphatase has been studied in the decidua and foetal membranes of the rat throughout pregnancy.

2. During the first 4 days the enzyme is present in the uterine epithelium, endothelium and leucocytes. From the 5th to the 11th days it is found in large amounts in the decidua. From the 11th day until full term it is present in

large amounts in the placenta and yolk-sac. The enzyme disappears from the uterine epithelium on the 5th day and reappears on the 13th day.

3. During the stages of cellular proliferation and differentiation the enzyme is predominantly nuclear in distribution.

4. Fully differentiated cells engaged in the transference of materials to and from the foetus show a heavy concentration of the enzyme in their peripheral cytoplasm.

5. The enzyme is least active in regions of the decidua, trophoblast and metrial gland engaged in the storage of glycogen.

6. Support is given for the hypothesis of Szendi and of Selye & McKeown that the glycogen cells of the spongy zone of the placenta have their origin in the metrial gland.

7. The so-called hypertrophic endothelium of the retro-placental maternal blood vessels is probably of trophoblastic origin.

8. The evidence is against Mossman's view that the placenta of the rat becomes haemo-endothelial in type late in pregnancy.

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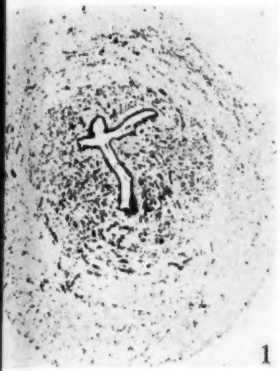

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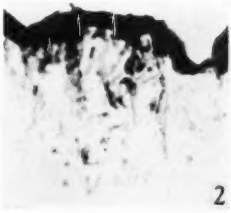

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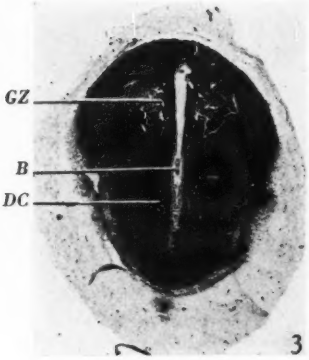

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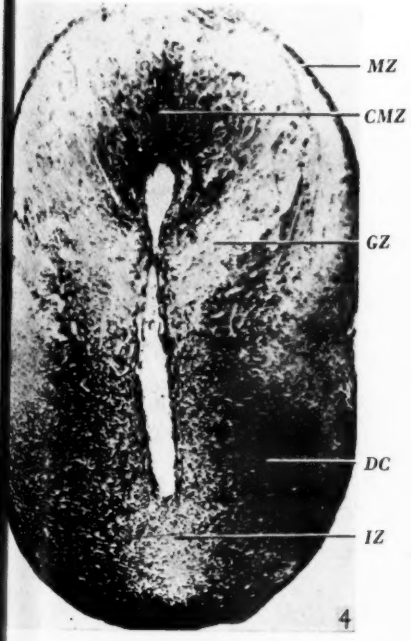

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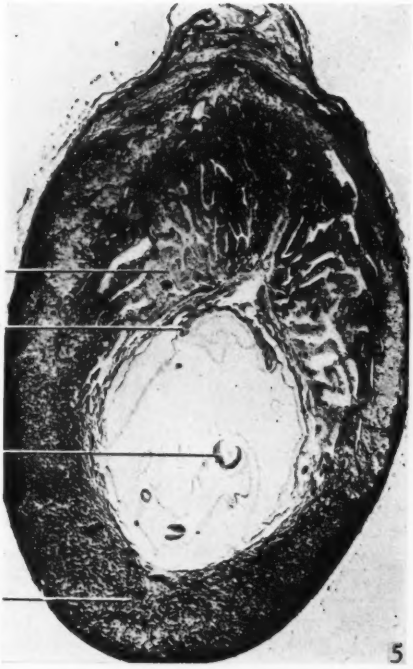

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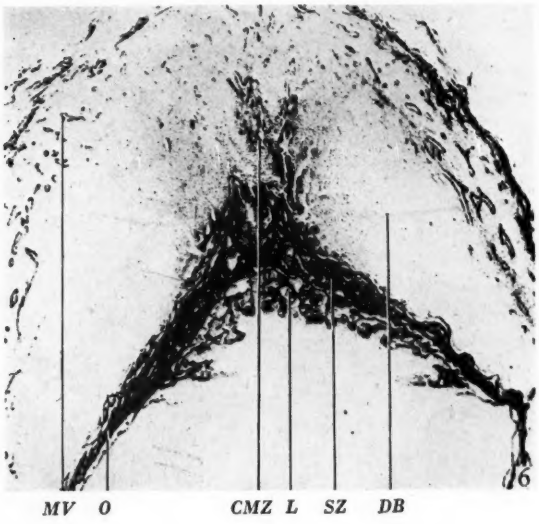
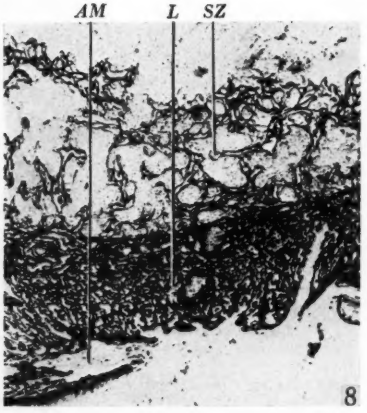
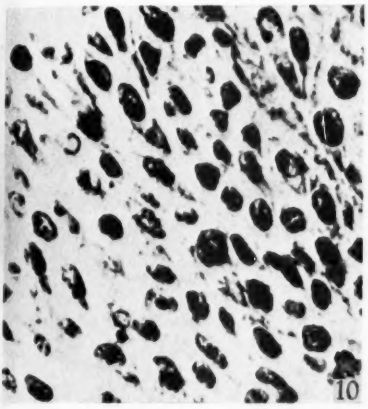
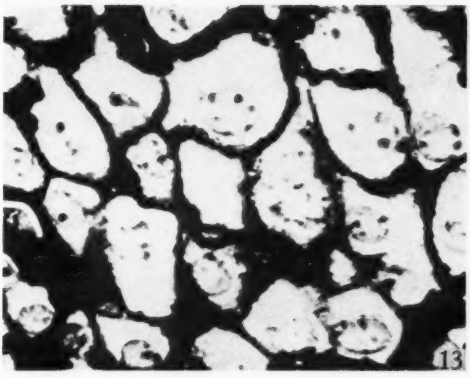
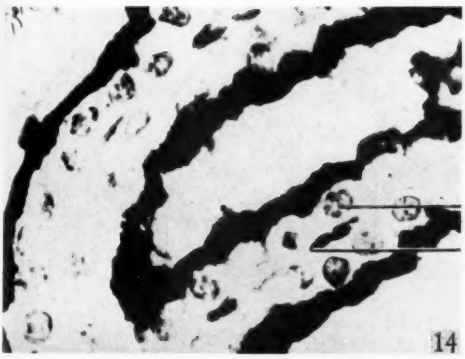


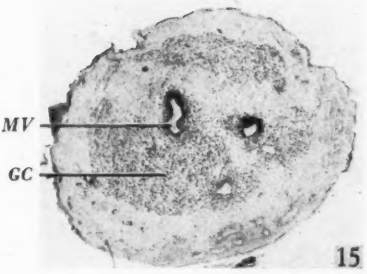


PRITCHARD—THE DISTRIBUTION OF ALKALINE PHOSPHATASE IN THE PREGNANT UTERUS OF THE RAT









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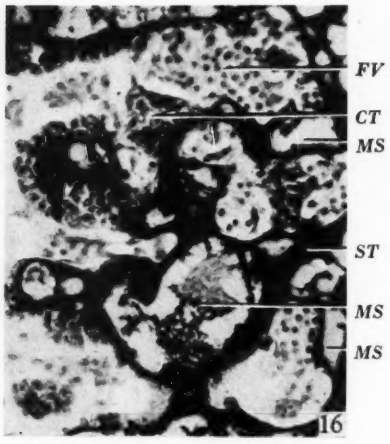
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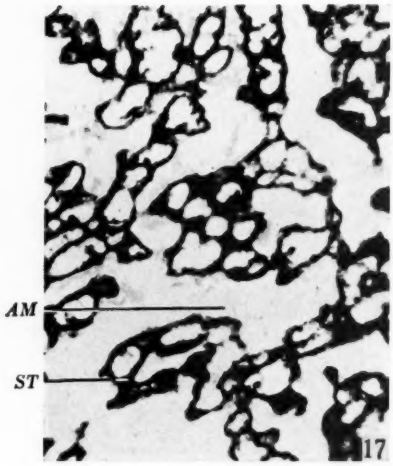
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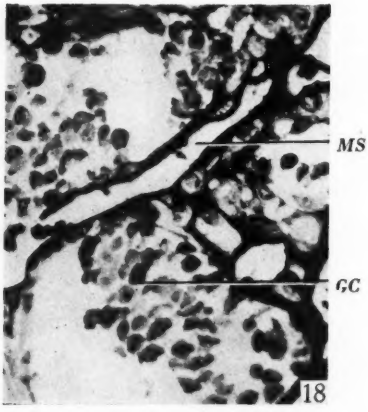
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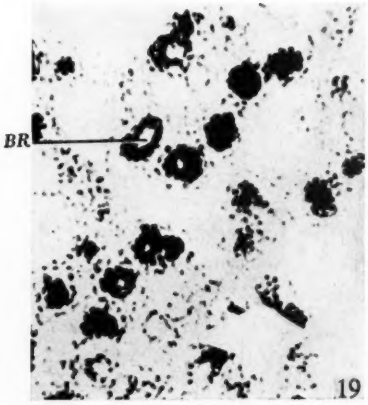
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EXPLANATION OF PLATES

All sections are stained solely for alkaline phosphatase, except for those shown in figs. 8, 15 and 18 in which glycogen has also been stained with Best's carmine.

PLATE 1

Fig. 1. Transverse section of uterus at the 1st day of pregnancy. ×8·3.

Fig. 2. Epithelium from part of section shown in fig. 1, showing intense staining of distal cytoplasm. ×700.

Fig. 3. Transverse section of uterus at the 5th day. The decidua is intensely stained, but only blood vessels stain in the muscularis. ×8·3.

Fig. 4. Transverse section of uterus at the 8th day. Only decidua stained. Contrast the intense staining of the central mesometrial zone, decidua capsularis and marginal zone, with the light staining of the glycogenic and implantation zones. ×8·3.

Fig. 5. Transverse section of uterus at the 10th day. In addition to the decidua, the trophoblast and the neural tube are stained. ×8·3.

PLATE 2

Fig. 6. Part of transverse section of uterus at the 11th day. Contrast the intense staining of the trophoblast with virtual absence of staining in the decidua basalis and allantois. ×8·3.

Fig. 7. Part of transverse section of uterus at the 17th day showing intense staining of the placenta and inner wall of the yolk-sac. ×8·3.

Fig. 8. Part of transverse section of uterus at the 15th day. Note the intense staining of the trophoblastic syncytium, both in the labyrinth and spongy zone. The glycogen cells of the spongy zone, stained with Best's carmine, appear as fine dots. ×8·3.

Fig. 9. Longitudinal section of foetus and placenta at the 19th day showing intense staining of the skeleton and glandular tissues, as well as of the placenta and yolk-sac. ×4·8.

PLATE 3

Fig. 10. Part of decidua capsularis at the 5th day showing intense staining of nuclei. ×594.

Fig. 11. Part of decidua capsularis at the 6th day showing intense nuclear staining combined with diffuse cytoplasmic staining. ×594.

Fig. 12. Part of inner wall of the yolk-sac at the 13th day showing intense nuclear staining with beginning distal cytoplasmic staining. ×594.

Fig. 13. Part of decidua capsularis at the 8th day showing intense staining of the peripheral cytoplasm with minimal nuclear staining. ×594.

Fig. 14. Parts of two yolk-sac villi at the 16th day showing intense staining of the distal cytoplasm with light to moderate nuclear staining. ×700.

Fig. 15. Transverse section of metrial gland at the 18th day showing two maternal blood channels with heavily stained lining cells. The glycogen cells, stained with Best's carmine, appear as fine dots. ×5·9.

PLATE 4

Fig. 16. Part of the labyrinth at the 12th day showing intense staining of the syncytio-trophoblast with moderate staining of the cytotrophoblast and foetal vascular endothelium and red cells. ×174.

Fig. 17. Part of the labyrinth at the 16th day, showing intense staining of the syncytium but absence of staining from the foetal blood vessels and mesenchyme. ×174.

Fig. 18. Part of the spongy zone of the placenta at the 15th day showing intense cytoplasmic staining of the trophoblast lining the maternal blood sinuses. Glycogen (grey) has been stained with Best's carmine. ×174.

Fig. 19. Part of the developing lung at the 19th day showing intense staining of the entodermal lining of the bronchi. ×149.

ABBREVIATIONS

AM allantoic mesenchyme
B blastocyst
BR bronchus
CA central artery
CMZ central mesometrial zone
CP chorionic plate
CT cyto-trophoblast
DB decidua basalis
DC decidua capsularis
EN entodermal nucleus
FE foetal endothelium
FV foetal blood vessel
GC glycogen cells
GZ glycogenic zone
IZ implantation zone
L labyrinth
LG lung
LV liver
MS maternal blood sinus
MV maternal blood vessel
MZ marginal zone
NT neural tube
O omphalopleure
SG salivary gland
ST syncytio-trophoblast
SZ spongy zone
YS yolk-sac

THE REGENERATIVE CAPACITY OF CARDIAC MUSCLE

By R. G. HARRISON
Department of Human Anatomy, University of Oxford

It appears from the work of a number of authors (Fleischer & Loeb, 1910; Christian, Smith & Walker, 1911; Heller, 1914; Karsner & Dwyer, 1916; Collier, 1922; Warthin, 1924; Bright & Beck, 1935; Macmahon, 1937; Moritz & Atkins, 1938; Mallory, White & Salcedo-Salgar, 1939; King, 1941) that there are still differences of opinion concerning the degree of regeneration occurring after damage to cardiac muscle. The following is an account of the findings after diathermy lesions to the rabbit's heart.

In six rabbits the right ventricle was injured by the application of a diathermy needle for 10 sec., causing an area of necrosis 3–4 mm. wide. The animals were killed 24 hr., 3, 5, 7, 9 and 14 days after operation.

At 24 hr. after operation there was necrosis at the site of injury and in a large surrounding area.

At 3 days there was a central core of necrotic cells surrounded by a zone of muscle-cell detritus and fibroblasts containing monocytes, macrophages, lymphocytes and polymorphs.

At 5 and at 7 days the central necrotic zone was smaller and the surrounding zone correspondingly larger. The surrounding zone was composed almost entirely of fibroblasts with hardly any leucocytes. It was being invaded by capillaries from the adjacent undamaged tissue.

At 9 days the central core was largely replaced by new blood vessels and collagenous tissue which had completely invaded the surrounding zone.

At 14 days there was little change from the condition at 9 days. There was very little necrotic muscle left, but on the other hand there was no attempt at regeneration of muscle.

In view of Le Gros Clark's findings (1946) the same type of lesion was made in the gracilis muscle of a rabbit as had been made in the heart (i.e. with a diathermy needle). Ten days after such an injury the gracilis showed clear evidence of regeneration, though there was a large amount of fibrous tissue which appeared to be hindering the growth of the new muscle fibres.

It thus seems that in the rabbit cardiac muscle has not the same regenerative capacity as general somatic muscle.

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NOTICES OF BOOKS RECEIVED

Ellis's Anatomy. By J. A. KEEN. (Pp. xv + 487; 216 figs.; demy 8vo; 42*s.* net.) Capetown: Stewart Printing Co. (Pty.), Ltd.; London: John Murray. 1946.

This is the well-known *Ellis's Demonstrations of Anatomy* revised and edited by Dr Keen of the University of Capetown, and with a foreword by Lord Addison, the editor of the last (12th) edition. The book is arranged on a regional basis, and contains, among other illustrations, much-reduced copies of the well-known *Illustrations of Dissections* by Ellis and Ford. There is an index of 37 pages.

Illustrations of Regional Anatomy. By E. B. JAMIESON, M.D., 7th ed. (320 Plates; in VII parts; pott 4to; 75*s.* net.) Edinburgh: E. & S. Livingstone, Ltd.

These are the now well-known Plates based on the diagrams used by Dr Jamieson to illustrate his University lectures on Regional Anatomy. A preface to this edition is included in each of the VII parts and explains the history of the series and the alterations made in this edition. Each part contains a list of contents and an index.

The Child's Lung; Developmental Anatomy, Physiology and Pathology. By STEFAN ENGEL, M.D. (Pp. viii + 332; 283 figs.; demy 8vo; 40*s.* net.) London: Edward Arnold & Co.

The book is divided into two Parts: Part I, pp. 1–150, deals with the bronchial tree and bronchial glands, and Part II, pp. 151–324, with the lung. As the subtitle implies, the developmental anatomy, physiology and pathology of the parts of the child's lung are discussed. The developmental anatomy described is almost entirely postnatal. 'The author has once more turned deliberately from functional to anatomical methods.' Great use has been made of the study of serial sections and of reconstructions. Pathology occupies more than half of Part I and one-third of Part II. There is a list of 128 references.

We are asked by the publishers to say that the price of Professor Ruggles Gates *Human Genetics* is now £3. 15*s.*

Anatomical Society of Great Britain and Ireland

PROCEEDINGS

November 1946 — *February* 1947

April 1947 — *June* 1947

RECORDED BY

W. F. HARPER

PROCEEDINGS OF THE ANATOMICAL SOCIETY

NOVEMBER 1946

The Annual General Meeting of the Society, for the Session 1946–47, was held at 2.30 p.m. on Friday, 29 November 1946, in the Anatomical Department of Guy's Hospital Medical School, London, S.E.1, the President (Professor J. C. Brash) in the Chair.

Dr J. Whillis presented a communication on 'The palate and tongue in snoring', which was illustrated by a cinematograph film.

Observations were made of the positions of the tongue and palate during nasal breathing, oral breathing and snoring. In breathing through the nose with the mouth either opened or closed the palate is tensed and held forward and the tongue overlaps the palatoglossal arch where it passes medially towards the uvula. During mouth-breathing the tongue drops until it is clear of the palatopharyngeal arch and the palate is raised slightly.

During snoring the tongue lies in a position intermediate between the palatoglossal and palatopharyngeal arches. The anterior part of the soft palate is tense and almost immobile. The snore is produced by a rapid vibration of the relaxed palatopharyngeal arch. The tongue is often asymmetrical, so that the snore occurs only on one side.

It is impossible to snore if the tongue is artificially retracted so as to clear the palatopharyngeal arch.

Snoring usually occurs with the mouth open, and the vibration of the palato-pharyngeal fold allows some air to pass through the mouth as well as through the nose. When snoring occurs with the mouth closed, the resonance is altered, but the mechanism is the same, residual air being sucked from the mouth into the pharynx.

Professor T. B. Johnston said that he had watched the movements described by Dr Whillis through a special apparatus which allowed the observer to see what was happening when the mouth was closed, and he was convinced that Dr Whillis's account of the mechanism of snoring was correct.

In a reply to the President who congratulated him on this work Dr Whillis said that a cinematograph film of the palate in natural snoring could not be made, as it would be impossible to get the view.

Professor M. A. MacConaill read a paper on 'Staining of the central nervous system with lead-haematoxylin'.

Mallory (*Amer. J. Path.* 1936, **12**, 569–71) has described a method of staining axis cylinders with haematoxylin after mordanting with lead chloride: the procedure is long and somewhat complex. The following method works after fixation of material in formalin, and takes only some 6 hr. from start to finish. The stain is prepared by mixing 2 g. of haematoxylin (in 10 c.c. of 95 % alcohol) with 100 c.c. of a 'stabilized lead solution', and making the mixture up to 200 c.c. by adding tap water. The mixture is filtered and used after half an hour. Paraffin sections are carried down to water in the usual way, and the slides are immersed in the staining solution for 5 hr. They are then washed in tap water, and mounted in balsam or, preferably, Clarite. Nerve fibres are stained dark blue, boutons terminaux are clearly shown, Nissl granules and the processes of all nerve cells are clearly marked, and neuroglia cells are left white. The method is applicable to post-mortem human material, as well as to fresh animal tissue.

The 'stabilized lead solution' is prepared as follows. Take a 5 % solution of lead nitrate in tap water. Add an equal volume of a saturated solution of ammonium acetate, also in tap water. Filter. Add 2 c.c. of 40 % formaldehyde solution for every 100 c.c. of the filtered solution. This solution will keep for many days.

The stain should be used immediately, as the haematoxylin is oxidized fairly rapidly. Batches of slides can be stained at a time, and the results are absolutely uniform. The time of staining can be prolonged if the worker is more interested in fibre groups than in synaptic fibres.

A full account of the method is to be submitted for publication in the *Journal*.

Professor GOLDBY asked whether the method described could be used for staining the peripheral nervous system; whether, for instance, it was specific for nerve fibres as opposed to connective tissues. Professor MACCONAILL replied that the method was promising in muscle preparations in which the finer nerves were deeply stained and the motor nerve endings were demonstrated. As applied to skin the results were disappointing, but this may have been due to difficulties in cutting the sections.

Dr F. P. REAGAN read a paper on 'The later morphogenesis of the quadruped heart'.

A series of developmental stages, particularly of the mole, was described. The so-called 'recession' of the valves of the right horn of the sinus venosus into the 'thoracic vena cava inferior posterior' was specially detailed. Evidence was presented that the structure generally described in quadrupeds as a greatly attenuated vena cava is in part an attenuated caudal horn of the pars dorsalis of the right atrium.

Dr FRANKLIN congratulated Dr Reagan on his detailed exposition of this problem.

Professor LAMBERT ROGERS delivered a communication on 'Some observations on the circle of Willis'.

The problem as to whether the circle of Willis is a distributor station for the cerebral blood supply, fed by the carotid and vertebral streams and giving off effluents to the brain, or merely an anastomosis between the vessels of these systems, comparable, say, with the anastomoses around the elbow and the knee, was considered. In an endeavour to answer this question, evidence has been accumulated from three sources, viz. (i) anatomical, (ii) clinical and (iii) experimental.

Investigation of many specimens has shown that the circle is only rarely defective and is almost always a closed ring, albeit frequently a very irregular or uneven ring, as the communicating arteries may be only thread-like connexions between the larger vessels.

Experiments on the cadaver have shown that coloured substances injected into one carotid artery remain for the most part in that artery or its branches.

Radio-opaque substances, e.g. thorotrast and diotrast, produce similar results in the living subject.

Pressure and volume changes following occlusion of a main affluent, such as one carotid, were studied in a working-scale model of the circle.

It is concluded that the circle of Willis is not a distributor station or booster mechanism on the cerebral blood supply, but merely an anastomosis between the main arterial trunks.

Dr WEDDELL said that Shorstein had shown that there was a good functional anastomotic circle at the base of the human brain. It was very uncommon for hemiplegia to develop after tying one common carotid when the arteries were otherwise normal. In his experience bleeding from the anterior ethmoidal artery could continue after the common carotid of the same side had been tied, and such bleeding could only be by way of a functional anastomosis.

Professor LE GROS CLARK commenting on the importance of the subject said it was very difficult to arrive at a conclusion regarding the functional significance of the

anastomosis. Clinically, tying of the common carotid was said to give rise to a 50% chance of hemiplegia, but he thought that such cases were grossly abnormal, since there was already cerebral anaemia from aneurysm or haemorrhage. Complete absence of one of the components of the circle might occur occasionally; the posterior communicating artery had been found absent in three cases out of 200.

Dr WEDDELL remarked that Shorstein recorded the absence of the posterior communicating artery in two out of 1200 cases.

Dr WALLS said that he had seen the apparatus described by Professor Lambert Rogers working, and the results were similar to the arteriograms produced clinically. If the calibres of the tubes could be varied as the vessels in the living subject, the model might give additional information.

Professor LAMBERT ROGERS replied that he had tried this by varying the calibres of the tubes representing the anterior and posterior communicating arteries, with some effect on the pressures recorded.

Professor LE GROS CLARK remarked further, that a study of the comparative anatomy of the region might throw some light on the problem. In mammals a rete mirabile often occupied the place of the communicating arteries, and this would perhaps slow up the circulation rather than allow for an impeded flow.

Professor BOYD said that he thought the circle must be a mechanism for distribution, for as many animals lack an internal carotid artery as possess one. The brain might be supplied by the external and internal carotids together with the vertebral arteries, or by any two, or sometimes by only one of these.

Professor CAVE considered that the available evidence from comparative anatomy favoured Professor Lambert Rogers's contention that the circle was merely an anastomotic, and not an equalizing mechanism. Though in the generality of mammalian species, the circle was complete, the anterior communicating artery was frequently a mere thread. The basilar artery was normally absent in certain forms (e.g. *Phocaena*). In the avian brain (emu, ostrich, penguin and macaw) the circle was incomplete both anteriorly and posteriorly, and the basilar artery was derived directly from the internal carotid. The remarkable incompleteness of the circle in the highly organized avian brain appeared to be inconsistent with any imputed distributor function.

The PRESIDENT, thanking Professor Lambert Rogers for his paper, suggested that the anastomosis was probably not comparable to that around the knee but was possibly nearer to that of the embryonic arteries round the edge of the placenta.

Dr W. BRANDT read a paper entitled 'Experimental production of a living artificial limb in the amphibian embryo'.

A living artificial limb was constructed from structures which normally do not develop at the area of the limb. The operation was performed in the tail-bud stage of the *Amblystoma* embryo (stage 36, Harrison). A large piece of ectoderm with underlying mesenchyme was cut from the posterior area of the back of the donor embryo. The excised ectoderm was folded upon itself under water and a long tube of ectoderm produced. A wedge-shaped piece was cut from the distal end of this tube, allowing both the outer edges to fold upon themselves. In this way two finger-like appendices of the ectodermal tube were produced. Two 'fingers' only were constructed in this experiment. The notochord was used for the construction of the skeletal element of the limb, this being the only skeletal element of the embryo available at this stage. A piece of the notochord was cut from the same donor embryo and put into the mesenchymal core of the ectodermal tube. The limb bud of the host was excised and the artificial limb transplanted into the deeper tissues in this area. The notochord developed well in the mesenchymal core, and muscle fibres from the shoulder girdle of the host embryo grew towards it. Within the implanted limb an imperfectly

developed humerus was formed, but all distal parts of the new limb of the host were absent. The notochord was not attached at the shoulder-girdle of the host, but came to lie on its dorsal aspect. This area had grown obliquely outwards as the result of pressure of the implanted notochord which prevented the normal direction of growth of the shoulder-girdle. The artificial limb was completely fixed. It is claimed that this is the first living artificial limb to be produced.

The PRESIDENT congratulated Dr Brandt on his remarkable extension of the technique of experimental embryology, but thought 'artificial' the wrong word in this connexion. He hoped that it would be possible for Dr Brandt to continue this work, and produce more perfect limbs later.

Dr R. G. HARRISON read a paper on 'The effect of adrenalectomy on the uterine weight of spayed immature rats'.

The uterine weight of spayed and adrenalectomized immature rats is greater than that of rats which have only been spayed. The histological appearance of the body of the uterus of the former is normal, while that of the latter shows atrophic changes. It is suggested that the adrenal cortex in the spayed immature rat exerts an influence on the uterus which is the cause of the regressive changes observed after spaying.

Incidental observations made on the weights of adrenals and uteri show that there is a definite average adrenal-weight/body-weight relationship in normal and spayed immature rats, while no such uterine-weight/body-weight relationship exists.

Professor LE GROS CLARK commented on the extreme difficulty in deciding an apparently straightforward point such as the existence of a difference between animals subjected to spaying as opposed to spaying and adrenalectomy. The most striking evidence was histological and this fortunately could not be attacked on statistical grounds, for uterine epithelium was either definitely atrophic or relatively normal.

Dr PRITCHARD asked whether the tallness of the uterine epithelium suggested that oestrone was still circulating.

In reply Dr HARRISON said that some substance similar to oestrone might still be produced in the organism after spaying. He hoped to continue his experiments and reach a definite result.

Dr G. WEDDELL delivered a communication on 'The Anatomy of pain sensibility'.

The nerve terminals which subserve pain sensibility in normal human skin are of a single morphological type. However, Lewis and subsequent workers have demonstrated that two types of pain can be aroused from the skin, 'fast' pain and 'slow' pain. In connexion with this observation, histological evidence is presented that pain terminals in human skin are connected with two types of nerve fibres, medullated and non-medullated. The terminals subserved by the medullated fibres are situated just beneath the epithelium, whilst the non-medullated fibres subserve terminals which are situated more deeply, and which are in relationship to the cutaneous blood vessels.

The PRESIDENT congratulated Dr Weddell on this important addition to his previous work on the skin.

Professor MACCONAILL commented on the clear demonstration that one type of nerve ending could be served by two kinds of fibres. Similar terminations were found in joints and the slowly aroused long-lasting type of pain was typical of these structures.

Dr D. C. SINCLAIR read a paper entitled 'Observations on the central pathways transmitting the sensation of pins and needles'.

Following the compression of a limb by a sphygmomanometer cuff for 10 min. or more, a number of clearly defined sensations, usually referred to collectively as 'pins and needles' are produced in normal subjects. The central pathway of some of these

sensations was ascertained as the result of examining patients with spinal cord lesions and the nature of the sensations is discussed.

Professor YOFFEY asked whether Dr Sinclair had any information on the pathways taken by tickling sensations, or on the point at which the changes which he had investigated had taken place, and whether they were due to anoxaemia at the nerve endings or to compression of the nerve trunk.

Dr SINCLAIR replied that he had no information on the pathways taken by tickling or itching sensations. The site of compression tingling was under the cuff of the apparatus he employed, while release pricking was probably due to obstruction of the venous return leading to the accumulation of chemical metabolites which made the peripheral nerve endings hypersensitive.

Professor YOFFEY suggested that the sensation from a direct blow on a nerve could not be due to an accumulation of metabolites. Dr SINCLAIR answered that 'pins and needles' from a direct blow was a different kind of sensation, distinct from that of 'release pricking'.

In answer to Dr PRITCHARD who commented on the hot and cold sensations associated with 'pins and needles' Dr SINCLAIR said that such sensations had been observed in his subjects, but they were inconstant.

Dr WHILLIS asked whether any deductions could be drawn from a study of patients suffering from dissociated anaesthesias due to lesions of the thalamus.

Dr SINCLAIR replied that in a case in which there was insensitivity to pinprick, though touch sensitivity was normal, compression tingling and release pricking were both absent.

Dr WEDDELL inquired if ischaemic pain had been studied, and Dr SINCLAIR mentioned a case in which the contractions made by the patient on the affected side were stopped by muscular stiffness and not by pain.

Professor WEST remarked that in his experience the sensations following the application of a sphygmomanometer were not referred to the skin, but to the deeper, possibly vascular structures, and they were 'bursting' in quality.

Dr SINCLAIR answered that this 'bursting' sensation was quite different from those he had investigated, and that it was not found in experienced subjects. His patients always referred their sensations to the surface of the skin or just beneath it, never deeper.

Drs J. T. AITKEN and M. SHARMAN and Professor J. Z. YOUNG read a paper entitled 'Effect of peripheral connexion in maturation of nerve fibres'.

Regenerated nerve fibres which are not allowed to reach the periphery remain small. In the case of a muscle nerve, contact with muscle causes reduction in number and increase in diameter of the regenerating fibres. Muscles which have been tenotomized are less effective than normal muscles in controlling the maturation of the nerve. Sensory nerve fibres also mature only if contact is made with the periphery. The nature of this effect which travels up nerve fibres is not known.

Dr PRITCHARD observed that the blood vascular system may play some part and suggested a possible further control of the experiments by cutting the nerve twice.

Professor YOUNG replied that the blood supply had not been controlled and that he thought it was unlikely that the observed effects were due to vascular changes.

Professor YOFFEY was told that it was unknown how far up the nerve the effect of the peripheral connexions might spread.

Dr J. JOSEPH (introduced by Professor J. Z. YOUNG) read a paper entitled 'Effect of section of non-medullated nerve on the cells of the peripheral stump'.

After division of the anterior mesenteric nerve of the rabbit, almost wholly composed of non-medullated fibres, there is no multiplication of the cells of the peripheral stump. The multiplication of Schwann cells which occurs during regeneration

of a somatic nerve after section must therefore be due either to the collapse of the fibres of large diameter or to the degeneration of myelin.

Dr WYBURN asked whether the cells observed were all Schwann cells or included macrophages, as it was usually believed that degenerating axons were removed by macrophagic action.

Dr JOSEPH replied that he had considered this point but had not differentiated the macrophages.

Dr WEDDELL remarked that in preparations stained with methylene blue the fibres and the cells of Schwann and Remak were differentiated, but no multiplication of the Schwann cells or invasion by macrophages was observed.

Professor LE GROS CLARK said that after the removal of the olfactory bulbs there was no invasion of the olfactory epithelium by macrophages, but remnants of the degenerating olfactory cells were ingested by the supporting cells.

Professor YOUNG remarked that following nerve section there may be some multiplication of the Schwann cells, but not to more than twice their original number.

Dr D. H. LODWICK EVANS (introduced by Professor J. Z. YOUNG) read a paper on 'Endings produced by somatic nerve fibres growing into the adrenal gland'.

Somatic nerves were joined to the great splanchnic of rabbits, and the adrenals examined 100–200 days later showed very large bulbous endings. The central stump as well as the peripheral has therefore an effect in controlling the diameter of regenerating nerve fibres. An interesting feature was that many fibres escaped from the bundles passing through the cortex and made endings among the cortical cells.

Dr AITKEN asked if there was any predilection of the ingrowing nerves for the outer zone of the suprarenal cortex.

Dr EVANS replied that that zone was remarkably free from nerve endings.

Professor LE GROS CLARK congratulated the authors of the last three papers on their most impressive work, adding that he was interested to see the progress in the work done under the direction of Professor J. Z. Young.

DEMONSTRATIONS

Guy's Hospital Medical School:

(1) M. A. MACCONAILL: Staining of the central nervous system with lead-haematoxylin.

(2) F. P. REAGAN: Dissections of the hearts of embryonic insectivora and chiroptera.

(3) F. BRANDT: Specimen of human siren.

(4) A. E. BARCLAY, P. DANIEL, K. J. FRANKLIN, M. M. L. PRICHARD & J. TRUETA: Renal vascular studies.

FEBRUARY 1947

An ordinary meeting of the Society, for the Session 1946–47, was held at 3.0 p.m. on Friday, 21 February 1947, in the Medical School, King's College, Newcastle-upon-Tyne, the President (Professor J. C. BRASH) in the Chair.

The PRESIDENT delivered a communication entitled 'Neuro-vascular hila of muscles: upper limb'.

A detailed study of the points and mode of entry of nerves and arteries into the muscles of the limbs was made in collaboration by the Anatomy Departments in the Universities of Edinburgh, Aberdeen and Leeds, and (lower limb only) Glasgow. The primary objects were to check and extend previous observations on the points of entry of the motor nerves and, in particular, in relation to war injuries, to determine to what extent the principal blood-supply of the muscles is associated with the nerves, i.e. the constancy or otherwise of a 'neuro-vascular hilum'.

Levels of entry of motor nerves agree in general with the previous data of Cappell (incorporated by T. H. Bryce in Quain's *Anatomy*, 11th ed., 'Myology', 1923) and of Linell (*J. Anat.* 1921); and they are in conformity with the illustrations of 'Motor points in relation to the surface of the body' published by R. W. Reid (*J. Anat.* 1920).

The mode of nerve-entry to individual muscles (inter-fascicular or trans-fascicular) agrees with Schwalbe's original statement (1897) that this depends on the form and dimensions of the muscle; but the variations in level of entry and in its situation in relation to the muscle as a whole make it doubtful whether his 'Gesetz des Muskelnerveneintrits', i.e. that the nerve enters at the 'Geometric centre' of the muscle, is more than generally applicable. In general, the situation of nerve-entry or the intramuscular subdivision of the nerve after its entry and the arrangement of the intramuscular plexus peculiar to each muscle (Frohse, 1898) appear to depend on facility of even distribution rather than on avoidance of drag during contraction, though the two things may well be associated.

The same principles appear to govern the entry of the main blood vessels of a muscle and in many cases, though by no means invariably, these do have a common entrance with the nerves or enter very near them. In some muscles a definite 'neuro-vascular hilum' occurs much oftener than in others; in general, a muscle of the upper limb exhibits a 'neuro-vascular hilum' in over 50 %, in some muscles it is over 80 %, and in at least two (Latissimus dorsi and Biceps) it is over 90 %. In most of the muscles there are subsidiary arteries entering at various points but usually related to the periphery of the muscular substance close to bony attachments or tendinous endings.

The presence of a principal artery and the number and situation of subsidiary vessels in any individual muscle may have a bearing on the efficiency of its blood supply in relation to the inadequacy of intramuscular anastomoses shown experimentally by Le Gros Clark and Blomfield.

Dr Hill asked to what extent, if any, the nerve and blood supply of muscles as studied by Professor Brash had been found to give indications of the morphological constitution of the muscles, with special reference to parts lost during phylogeny and which appear with more or less frequency in lower races of man. He mentioned the coraco-brachialis as an example of a muscle likely to have left indications of its former greater extent (i.e. coraco-brachialis brevis now lost and coraco-brachialis longus and superficialis now more restricted than formerly). Dr Hill also alluded to the statement made by Professor Brash, that the extensor indicis had two nerves and he suggested that one of them was the nerve of a lost belly originally passing to the middle finger.

In reply Professor Brash said that as regards blood supply the records for the coraco-brachialis were insufficient to make a definite statement. As for its nerve supply there was some constancy in the separate supply of the different parts, but the nerves tended to intercommunicate before finally entering the muscle. He agreed with the suggestion regarding the double nerve supply to the extensor indicis.

Professor West who asked if the 'geometric centre' corresponded with the 'neuro-vascular hilum' was told that this was so in most cases.

Professor Kirk, from the Chair, congratulated Professor Brash for his interesting practical communication and looked forward to a similar contribution on the lower limb.

Mr G. F. Rowbotham (introduced by Professor R. B. Green) read a paper entitled 'Pain pathways of the face and head'.

The types of operation carried out for the relief of trigeminal pain were indicated, e.g. blocking the trigeminal ganglion in its third or second divisions, section of the posterior nerve root, section of the first, second or third divisions of the nerve, division of the descending trigeminal tract in the medulla, removal of the sensory cortex of the face and bilateral pre-frontal leucotomy.

Pains of the head and face can be classified into three main groups, viz.:

(i) The pain of major trigeminal neuralgia. Nerve block or nerve section in this type of case gives complete relief from pain with paraesthesia.

(ii) Pain typified by migraine. The evidence in these cases is that the pain is due to excessive spasm or dilatation of blood vessels in the head. In some of these cases the pain impulses appear to pass along the upper part of the trigeminal root. Thus pains arising in the autonomic structures in the head may pass by the so-called somatic pathway to the brain.

[The speaker here mentioned that so-called 'uterine pains' do not always arise in the uterus, but may be caused by disturbances in nearby structures or by displacement of the uterus.*]

(iii) Continuous burning pain in the jaw. Such pain is never relieved by root section, by tractotomy or by cervical sympathectomy. Such a case was described in which section of the sensory cortex also failed to give relief. Bilateral pre-frontal leucotomy cured the pain but left the patient mentally defective.

A final case of post-herpetic neuralgia was mentioned. Trigeminal root section and cervical sympathectomy failed to cure the pain, but an incision down to bone severing all the structures crossing the eyebrow gave relief.

If the facts are true then painful impulses can reach the brain from the 'trigeminal territory', other than through the posterior root of the trigeminus.

Professor YOFFEY inquired whether pains could start in the uterus itself or in autonomic structures themselves. Mr ROWBOTHAM replied that he supposed they could—his point was that a disturbance in the autonomic mechanism may cause pain by 'deforming' some nearby structure. Cheiropractors occasionally cure 'kidney pains' by 'pulling out' painful spasm of the quadratus lumborum.

Professor YOFFEY also asked whether sensory corticectomy would relieve pain in the face. Mr ROWBOTHAM replied that he had done only three cases and all had failed. He added that a case had been described in which corticectomy relieved pains of a phantom limb.

Dr WEDDELL stated that although the problem of pain is a large one there is a body of anatomical evidence accumulating which demonstrates that pain is subserved by specific terminals borne by specific nerve fibres which behave in the same manner as other somatic nerve fibres in respect of the 'all or none' law, etc. There is also evidence that a change in quality of pain sensibility results from a disturbance of the peripheral anatomical pattern of pain terminals. Normally the terminals of adjacent fibres interweave with one another, but in conditions where the quality of pain sensibility is altered, terminals are observed to be isolated. Dr WEDDELL thought that it was along such anatomical lines that our knowledge of pain, and particularly our ability to relieve pain in disease, would advance.

Professor F. KISS (introduced by Professor F. GOLDBY) read a paper on 'The blood-vascular system of the eyeball'.

The blood vessels of the eyeball were studied after injection with India ink through the ophthalmic artery in human (new-born) specimens and in albino rabbits.

In the iris the majority of the vessels are veins; a probable function is the warming of fluid in the chambers of the eye.

In the ciliary body two systems of vessels are found: large dilated vessels in the ciliary processes, and small fine vessels in the ciliary muscle. The former are probably concerned with the production, the latter with the absorption of the aqueous humour. Particulate matter or solutions injected into the anterior chamber find their way into the plexus of small fine vessels in the ciliary muscle. Both ciliary systems drain into the venae vorticosae of the choroid, but the fine ciliary plexus has also permanent

* See Yoffey in discussion.

anastomoses through special tortuous veins with the episcleral plexus. The canal of Schlemn is a simple collecting vein having T-shaped or oblique anastomoses with the episcleral plexus.

The blood circulation of the eyeball is concerned with four main functions: (i) nutritive functions, (ii) the production of humour, (iii) the absorption of humour, (iv) the maintenance of normal intraocular tension. It is a complex system in labile equilibrium controlled through the innervation of the ophthalmic artery. When the ophthalmic artery is abnormally dilated, the circulation in the eyeball is blocked except for the emergency anastomoses between the fine ciliary plexus and the episcleral plexus. This mechanism can account for the circulatory changes found in glaucoma.

Professor KIRK, in confirmation of the concepts presented by Professor Kiss, mentioned the ring of enlarged and distended episcleral veins at the corneo-scleral junction (anterior ciliary group), which was so noticeable a feature in the later stages of unrelieved glaucoma, and was, no doubt, indicative of a vascular engorgement of the ciliary body and ciliary muscle in these cases.

Professor YOFFEY asked whether there had been any measurement of the pressure with which the India ink was injected into the anterior chamber of the eyeball. It was an unusual phenomenon for India ink particles to pass through the walls of blood capillaries, and unless the ciliary capillaries were radically different from those elsewhere in the body, the passage of India ink into them was difficult to explain.

Professor KISS replied that the injection was done by means of hand syringe and that there was no control of the pressure. He could offer no explanation for the presence of India ink particles in the plexus of the ciliary muscle following injection of the anterior chamber.

Dr G. W. HARRIS read a communication entitled 'Significance of the zona tuberalis of the rabbit's hypophysis'.

In 1937 Dawson described a specialized region of the pars distalis in the hypophysis of the rabbit and cat which he termed the zona tuberalis. This structure has been studied in the rabbit by dissection and by histological examination. It would appear that the morphology of the zona tuberalis is determined by (*a*) the length and direction of the hypophysial stalk, and (*b*) the vascular pattern of the region, especially the form and position of the superior hypophysial arteries and the hypophysial portal vessels. The embryology and functional significance of this structure was discussed.

The PRESIDENT congratulated Dr Harris on his complete and convincing demonstration.

This paper will be published in full in the *Journal*.

Dr J. J. PRITCHARD read a paper entitled 'Glycogen and alkaline phosphatase in the pregnant uterus of the rat'.

The distribution of glycogen and alkaline phosphatase was studied histologically in the uterus of the pregnant rat, with the object of testing Wislocki & Dempsey's (1945) hypothesis (*Amer. J. Anat.* 77, 365) that cells containing alkaline phosphatase are always present between the maternal blood stream (source of carbohydrate raw material) and deposits of glycogen in the placenta and decidua.

Before implantation of the blastocyst no glycogen was found, and alkaline phosphatase was confined to surface and glandular epithelium, vascular endothelium and leucocytes. After implantation (6th day) newly formed decidual cells stained intensely for phosphatase, especially in the capsularis and separation zones. When glycogen began to be deposited in the basalis (7th day) phosphatase activity there rapidly declined.

After the 10th day glycogen and phosphatase were found chiefly in the foetal membranes. Phosphatase was present throughout the placenta, in the trophoblastic syncytium lining the maternal blood channels and in the villous part of the yolk sac.

Glycogen, on the other hand, accumulated chiefly in the spongy zone of the foetal placenta, and in the extension of this zone around the maternal blood sinuses of the basalis and mesometrium. There were small deposits in the foetal giant cells and villous epithelium of the yolk sac. In the last 5 days of pregnancy it was present throughout the labyrinthine syncytium.

On comparing the distribution of glycogen and phosphatase it was made clear that conditions in the giant cells, yolk sac epithelium and late labyrinthine syncytium supported Wislocki and Dempsey's hypothesis, but that conditions in the decidua were against it. In the spongy zone, although both substances were present, the greatest deposition of glycogen occurred where there was least phosphatase. It appears, therefore, that the hypothesis is not of universal application.

Lack of time prevented discussion of this paper.

Professor F. Kiss read a paper on 'Cell multiplication in peripheral ganglia'.

In 1932 the author demonstrated to the Anatomical Society of Great Britain and Ireland certain multipolar cells present in all peripheral ganglia, which could be stained by his prolonged osmic acid method. One to four days after section of the branches of any cranial nerve in young dogs, multiplication of these cells occurs in the corresponding ganglion (Gasserian, geniculate, jugular, etc.). A similar multiplication occurs in spinal ganglia after section of the rami communicantes or of intercostal nerves. Similar new cells are found in young human individuals after destructive pathological processes affecting the ganglia.

All peripheral ganglia contain both somatic and vegetative nerve cells, the vegetative cells in young individuals multiply after section of their peripheral branches, later in life they degenerate.

Professor Goldby remarked that in sections of the central nervous system he had observed similar cells to those described by Professor Kiss which stained more deeply but there was no evidence of cell division. Many of the appearances suggested fixative effects and he thought this should be investigated.

Professor Kiss replied that he had seen no variation in the effects following the use of different fixatives and, further, the appearances described were not observed in control experiments.

Dr Weddell said that he thought it would be dangerous to accept the statement that multiplication of cells occurs in these ganglia following the experiments described by Professor Kiss.

Professor Yoffey inquired if aseptic precautions had been taken during the operative procedures. It was well known that bacteria and toxins pass quickly along nerves and these might have produced the effects observed.

Professor Kiss replied that a most careful operative technique was employed and that there was no possibility of infection.

Dr Pritchard, who asked whether it might be possible that the ganglion cells were actually degenerating as a normal event, possibly speeded up by operation, was told that similar appearances had not been observed in control animals.

Dr Bacsich remarked that the cells described were probably healthy cells but further work would be required to elucidate their real nature and function. He suggested careful cell counts of the ganglia and the application of the colchicine method.

A communication from Drs G. M. Wyburn and P. Bacsich on 'An experimental production of heterotopic bone' was read by title. Bone homografts were implanted subcutaneously into six guinea-pigs and removed within 3–5 days. The host tissue was sectioned and examined after 21 days and in three of the animals new bone formation had occurred around the cavities formerly occupied by bone grafts. In

these three cases there was some infection. The nature of the host reaction to the graft in producing an ossifiable medium and of the bone homograft in providing the osteogenetic stimulus was discussed. It is thought that the release of the osteogenetic stimulus would be facilitated by increased enzymatic activity due to infection.

DEMONSTRATIONS

The Medical School, King's College, Newcastle-upon-Tyne:

(1) C. A. ERSKINE: Sections illustrating changes in the brain stem following intramedullary tractotomy.

(2) R. V. BRADLAW: Innervation of the teeth and jaws.

(3) F. KISS: The blood supply of the prostate gland.

APRIL 1947

An ordinary meeting of the Society, for the Session 1946–47, was held at 2.0 p.m. on Friday, 25 April 1947, at the London (Royal Free Hospital) School of Medicine for Women, 8 Hunter Street, Brunswick Square, London, W.C.1, the President (Professor J. C. BRASH) in the Chair.

Dr G. W. HARRIS read a paper on 'The neurohypophysis (posterior pituitary gland) as an organ of internal secretion'.

Views as to the function of the neurohypophysis have undergone much change in the past century. It has been thought to be a neuroglial structure, a nervous structure transmitting afferent stimuli to the brain, an anatomical pathway for hormones formed by the pars intermedia of the adenohypophysis (anterior pituitary), and lastly an endocrine organ in its own right. This last view is now finding more general acceptance, though only the antidiuretic factor has a proven hormonal status. Data gained from direct stimulation of the rabbit neurohypophysis or of its nerve supply in the stalk are presented and analysed in terms of the criteria of an endocrine organ.

Professor ZUCKERMAN asked if stimulation of the hypothalamus, or supraoptic nucleus directly, might produce results similar to those obtained by stimulation of the neurohypophysis.

Dr HARRIS replied that stimulation of various areas of the hypothalamus did not produce these results, provided the stimulating tip of the electrode is more than 0·5 mm. from the supraoptico-hypophyseal tract. Control experiments showed that the current spread is not more than 0·5 mm., for if the electrode tip is situated more than 0·5 mm. from the supraoptico-hypophyseal tract, either above, below, or on either side of it, secretion of the neurohypophysis does not follow stimulation. Stimulation of the supraoptic nucleus was not studied for the results of applying stimuli in this region are vitiated by an emotional response on the part of the animal, presumably due to concurrent stimulation of the visual pathway.

Professor W. E. LE GROS CLARK delivered a communication entitled 'Observations on the anatomy of the fossil Australopithecinae'.

As the result of facilities generously provided by Professor Dart and Dr Broom the speaker recently examined the fossil Australopithecine material in South Africa. In details of skull and dentition certain characters differ significantly from those of modern apes and make a remarkable approach to the Hominidae. These include the contour of the supraorbital region, the relationships of the frontal pole of the brain, the relatively weak development of the temporal ridges, etc. The hominid characters are paralleled by the limb bones. The available data indicate that the Australopithecine apes were capable of standing, and walking with an erect posture. Indirect

climatological evidence indicates that their habitat was probably rather arid and certainly not forested.

This study has made it clear that, as already emphasized by Dart and Broom, these extinct Primates are of quite outstanding significance for the consideration of problems of human evolution. Contrary to some opinions which have been expressed, they have no close affinity to the modern anthropoid apes.

Whether the fossils have an ancestral relationship direct or indirect to Homo sapiens can only be finally determined when their geological age is accurately known. The general opinion at present is that the deposits from which the fossils were derived are probably of Pliocene date.

Professor Wood Jones remarked that it was very difficult for him to discuss this communication as Professor Le Gros Clark, in spite of the alleged detailed resemblances to Homo sapiens, had not committed himself to a definite pronouncement regarding these fossils. He could not agree that Professor Le Gros Clark had 'demonstrated' the premaxillary-maxillary suture in his figure of a full-term foetus. This suture is never present in man. So far as could be inferred from the drawing of the incomplete fossil talus, it spoke for itself, as being from a pes with an abducted big toe and an inverted foot.

Professor Zuckerman said that much doubt attaches to the factual descriptions of the South African fossil apes that have been presented so far, and to the evolutionary significance attributed to them by Dr Broom and Professor Le Gros Clark. The anatomical descriptions, including that in Broom's big monograph, are inadequate because the material has not been properly compared with adequate existing anthropoid material. Such quantitative statements as are made in, for example, Broom's monograph, or the comparisons made by Professor Le Gros Clark, may be indicative of differences, but have little significance. Until we know to what extent the characteristics of the South African fossil apes fall outside the determinable variance of the corresponding characters in existing anthropoids, it is unwise to press the factual discussion further.

From the point of view of the phylogenetic significance that is attached to the material both by Dr Broom and Professor Le Gros Clark, one is again in a difficulty because it would seem, in particular from Broom's monograph, that the evolutionary ideas that are implied differ from views established in relation to modern studies of genetics and selection. While unconventional evolutionary views need not colour the factual statements of palaeontologists, they unfortunately appear to have done so in the present instance. For, by the author's own admissions, the facts about the South African fossil material have been stretched, presumably in order to conform with the view that the material is more human than ape-like, and in places to such an extent as to make them meaningless.

Professor Cave remarked that a personal study of the casts of the Australopithecine specimens was necessary before attempting any true assessment of the relationships of *Paranthropus* and *Plesianthropus* to both the extant African anthropoid apes and to man. In numerous anatomical features, the new fossils exhibited unmistakable similarity to the gorilla-chimpanzee form: in other respects they showed differences from the modern ape pattern which, though regarded by some as indicating an approximation to hominid status, were more reasonably interpreted as the mere morphological expression of phylogenetic primitivity.

Dr R. J. Harrison read a paper on 'Ovogenesis, follicular development and atresia in the ovary of the goat'.

Ovogenesis in the goat ovary occurs continuously throughout the oestrus cycle, with a maximum number of oocytes being produced during the last days of pro-oestrum. A striking characteristic of the goat ovary is the presence of polyovular follicles and

multinuclear ova, particularly in the ovaries of animals killed in pro-oestrum and oestrum. In several animals, killed during oestrum, 25–30 % of the total number of primary oocytes showed multiparity in some form. All the multiple follicles appear to degenerate within a few days of oestrum and no case of development of a multiple follicle beyond the primordial stage has been observed. The possible significance of such large numbers of multiple follicles in the goat ovary was discussed.

The subsequent development of the normal oocytes was described, both histologically and statistically, and the results compared with those from other ungulates. The part played by the theca interna cells in follicular development, atresia and in the formation of the corpus luteum was described.

Lack of time prevented discussion of this paper.

Professor W. J. Hamilton delivered a paper entitled 'Development of the egg of the golden hamster up to the stage of the blastocyst' based on work by himself and the late Dr D. M. Samuel.

In the *Journal of Anatomy*, **76**, 1943, Samuel and Hamilton gave a preliminary account of the cleavage stages of the living egg of the golden hamster. A detailed account of the measurements of the living eggs up to the blastocyst stage is now presented together with details of the histological appearances of the eggs during cleavage.

Unsegmented eggs were recovered from 12 to 24 hr. after mating, the two-cell stage from 25 to 46 hr., the four-cell stage 59–60 hr., the eight-cell stage 60–79 hr., and the blastocyst stage from 79 hr. until the time of implantation (approximately 110 hr.). Intermediate stages of five-, six- and seven-cell stages are found. The diminution of the amount of cytoplasm is discussed.

At the pronuclear stage the cytoplasm around the pronuclei shows a fine granular appearance; the more peripherally placed cytoplasm is irregularly striated. At the two-cell stage the cells do not divide synchronously. The spindles of division when formed are arranged at right angles to each other. The cytoplasm is finely granular in the central and peripheral parts of the blastomeres. A morula stage is not a feature of this animal. Soon after the eight-cell stage a blastocyst is formed. The cells of the trophoblast are more darkly stained than those of the inner cell mass.

Professor J. P. Hill congratulated the authors on the excellent photography of the living eggs and commented on the curious granular appearances seen in the ovum. The mode of attachment, he thought, bore certain resemblances to that found in the guinea-pig.

Mr H. F. Lunn read a paper on 'The inguinal canal in animals and man'.

The general disposition of the inguinal region in reptiles and mammals including man was described. The derivatives of the three primary layers of the body-wall musculature were traced in their relation to the canal in the types studied. An inguinal ligament is found in those forms which have a tendinous insertion of the external oblique muscle. The internal oblique muscle is attached more firmly to a fibrous arch over the ilio-psoas than to the inguinal ligament. The transversus muscle and its tendon are often adherent in part to the internal oblique and to the peritoneum. No evidence was found in the animals dissected, either fresh or after preservation, of a constant transversalis fascia. The various types of processus vaginalis were described in relation to the degree of closure of the neck.

The findings were compared with the current descriptions of the inguinal canal in man and with the appearances seen at operation. The author found that many students had wrong conceptions regarding the form of the inguinal canal in man: many believed that the spermatic cord passed medial to the pubic spine. He considered that this was probably due to faulty descriptions and teaching. Undue emphasis has been laid on the contribution of the inguinal ligament to this region

rather than that of the ilio-pectineal septum, and on the transversalis fascia rather than the tendon of the transversus.

Professor LE GROS CLARK said that he was not aware that he or anyone else taught that the spermatic cord passed medial to the pubic spine. He taught that it passed over it.

Mr LUNN replied that as a result of questioning students he had found that they, as a rule, believed it did pass medial to it, but he was unable to discover the source of their information.

Professor HAMILTON who asked if there was a descent of the testes in *Hyrax* was told that the testes in this animal were firmly fixed to the posterior abdominal wall below the kidneys and did not descend into the groin.

Dr FYFFE congratulated Mr LUNN on a careful comparative study of the inguinal region illustrating in certain animals generalities applicable to human anatomy. These generalities for the human, he said, are clearly given in a paper by McVay & Anson (*Anat. Rec.* 1939, **76**), and it would be preferable to teach students along these lines. However, we tend, for simplicity, to teach that the inguinal ligament is an entirety, centring important relations on it. But it is a complicated structure, and clinically the student later finds that it is not so clear-cut a landmark as his anatomical teaching led him to believe.

Drs E. GUTTMANN and R. E. M. BOWDEN read a paper on 'Some problems raised by the study of muscle biopsies taken from long-standing cases of anterior poliomyelitis'.

In the course of an investigation into the changes in human voluntary muscle due to denervation and reinnervation, material from long-standing cases of anterior poliomyelitis was examined. As would be expected, some of the muscle fibres showed signs of varying degrees of denervation atrophy, whilst in some cases there were also some normal fibres. In some instances the intramuscular nerve trunks contained fibres that were indistinguishable from regenerating axons; in one case where the muscle fibres showed extremely advanced atrophic changes, the nerve fibres appeared to be normal. These findings are of considerable theoretical interest, the tentative interpretation is that this provides some evidence of regeneration of nerve fibres. Should this prove to be correct, it raises problems of practical importance in the treatment and management of these patients.

Dr WEDDELL emphasizing the importance of this work said that the cases described provide us with very good evidence that a sprouting of nerve fibres into denervated muscle does occur. He further remarked that it had been shown that nerve fibres send out sprouts into denervated areas of the tongue.

Dr R. BARER read a paper entitled 'The swelling of striated muscle fibres in acids and alkalis'.

When teazed striated muscle fibres are treated with dilute acids, such as 0·2% lactic acid, colloidal swelling of the muscle substance occurs. This may result in spontaneous rupture of the sarcolemma with herniation of the muscle substance. At the same time the muscle nuclei becomes visible. In many cases the nuclei and striations can be seen to flow towards, and through, a sarcolemmal rupture. The striations often become very distorted on escaping from the sarcolemmal sheath. The flow of striations can be impeded by means of micromanipulation, the striations behaving as viscous deformable gels. Microdissection of the swollen muscle substance shows that it can be split readily into groups of striated myofibrils, but occasionally it is possible to break it into separate transverse discs. The effects of dilute alkalis are very similar to those of acids, but the nuclei remain invisible. If a fibre in which the nuclei have been rendered visible by acids is subjected to alkali, the nuclei disappear, but reappear on acidifying once more. This process is reversible and can be repeated

indefinitely. These results are discussed in relation to muscular fatigue and to work on the physiological effects of acids on muscular contraction.

Dr BOURNE remarked that in Dr Barer's slides depicting terminal herniation he noticed that on the fibres from which the muscle substance had been extruded there appeared to be structures resembling 'canals', and that among the extruded material there were spiral structures.

Dr BARER replied that the 'canals' were probably strands of connective tissue outside the muscle fibre. The spiral structures were actually the nuclei and they were frequently seen in this form.

Drs R. G. HARRISON and A. J. CAIN read a paper on 'Variations in the distribution of lipoids of the adrenal cortex'.

In the adrenal cortex of the rat there is considerable variation in the quantity and distribution of gross lipoid, lipine and lipoid substances colouring red and blue with nile blue. No corresponding variation in cholesterol is apparent. Suggestive evidence is presented for a rhythmic change in lipine content of the adrenal cortex.

Dr BOURNE inquired if the Schultz cholesterol test had been applied to the preparations and was informed that it had failed to demonstrate the variation.

Dr BAXTER remarked that in his experience the Schultz test was not very delicate. He had employed the alcoholic digitonin test for cholesterol, examining the sections beforehand to see if birefringence was present, then again after treating with alcoholic digitonin—the increase in birefringent material representing cholesterol made visible.

Dr SOSA asked if the authors had been able to locate any special type of lipoids in cytoplasmic structures, such as the Golgi apparatus and the mitochondria that have been considered by different authors as having a basic lipoidal constitution.

Dr HARRISON replied that they had not an answer to that question so far, but they were studying the Golgi apparatus in connexion with lipoids in the adrenal.

Drs D. V. DAVIES and T. MANN presented a communication on 'The function, anatomy and development of the "prostate" gland in the rabbit'.

Previous investigations have shown that mammalian semen contains fructose, originating in the seminal vesicles and providing nourishment for the spermatozoa by glycolytic breakdown to lactic acid (Mann, T., 1946, *Nature, Lond.*, **157**, 79; *Biochem. J.* **40**, 481). Most investigators maintain that the rabbit possesses no seminal vesicles. Despite this its semen has a large content of fructose (600 mg. %) and in this respect resembles that of animals such as the bull, ram, boar, guinea-pig, rat and man which possess well-developed seminal vesicles. The rabbit, however, has a large complex organ, sometimes referred to as the 'prostate', which has been described hitherto as composed of three glands, each with an independent urethral outlet (Leydolph, W., 1930, *Z. mikr.-anat. Forsch.* **19**, 285; Gerhardt, U., 1938, *Handb. vergl. Anat. d. Wirbeltiere*, **5**, 878). These glands are usually defined as 'Gl. seminalis', 'Gl. vesicularis' and 'prostate proper'. Fructose is found mainly in the Gl. vesicularis, although it is the Gl. seminalis which has been regarded usually as homologous to the seminal vesicle in other mammals.

In view of the inadequate evidence as to the origin and anatomy of these three glands, their gross anatomy and development were investigated.

In the adult rabbit the Gl. seminales open widely below into the single median Gl. vesicularis, which in turn opens into the urethra in common with the vasa deferentia. This description is at variance with that of Leydolph. In the 22-day embryo, bilateral diverticula develop from the Wolffian ducts. They communicate widely with these ducts and are separated medially by the Müllerian ducts. The Gl. seminales develop as proximal extensions from these diverticula, fuse in their central parts into a single sac, the glandula vesicularis, through the Müllerian ducts, so that the latter form a narrow strip on the anterior and posterior walls of the

common vesicle. This Müllerian duct epithelium is, however, soon excluded from the walls of the Gl. vesicularis by overgrowth of the more laterally placed Wolffian duct epithelium. Above and below this the bilateral diverticula fuse directly without the intervention of Müllerian ducts. The openings of the vasa deferentia, at first placed high upon the Gl. vesicularis, are rapidly transferred to its lower end on the 25th day of development.

The development of the 'prostate proper', paraprostatic glands (Gl. Cowperi superior) and Cowper's glands (Gl. Cowperi inferior) was also investigated.

The PRESIDENT, congratulating the authors, remarked on the excellence of their photomicrographs. In reply to his question as to whether anything corresponding to a uterus masculinus had been noted, he was told that no structure representing it is present in the rabbit.

Professor HAMILTON inquired whether sino-vaginal bulbs were present as such.

Dr DAVIES replied that a thickening of endoderm was present in the midline on the dorsal wall of the urethra and he considered this to represent the sino-vaginal bulbs.

Professor LE GROS CLARK remarked that oestrogenic stimulation experiments might prove interesting in the further investigation of this problem.

Professor A. Low delivered a communication entitled 'Freemartins'.

'Mart' is an old Scotch name for a cattle beast that did not breed, either a sterile heifer or a 'stot'. About Martinmas farmers slaughtered a Mart, salted it and put it in barrels for winter use.

John Hunter, the famous Scottish scientist and surgeon, whose dictum was 'Don't think, try', in other words, experiment, was told about freemartins by his houseman—Dr Edward Jenner. Hunter then acquired three freemartin heifers and kept them at Earl's Court. Unfortunately, he did not obtain the 'clenses'. On two points I was in doubt. The letterpress on the genitals of Wright's and Arbuthnot's freemartins did not correspond, and Dr D. Berry Hart figured and described a microphotograph of one of Hunter's freemartins showing spermatozoa, which I had never seen in the genital glands of freemartins.

With the permission of Sir Arthur Keith, who was then Curator of the Museum, I went to the Museum and studied Hunter's freemartin specimens. I had been using the second edition of Hunter's *Animal Oeconomy*, and on obtaining the first edition from the College Library found that the letterpress of these two freemartins had been transposed in the second edition. Further, the testis described by Dr D. Berry Hart was not from one of Hunter's specimens but from a hypospadiac ram. Then the technician who prepared the section pointed out where he took the specimen. We found Hunter's freemartin with its original number, 678, on the shelf of a neighbouring case.

Aberdeen Angus and Shorthorn freemartins are shown with their genitals and microsections of their sex glands; also twin 'clenses' showing communication between the arteries and veins. Keller and Tandler were the first to suggest that the internal secretion of the bull affected the genitals of the heifer. I have reliable evidence that five cases of Aberdeen Angus and Shorthorn freemartins were single calves, and from the Herd Books of these cattle there were not enough twins to account for the number of freemartins that were slaughtered.

In pigs freemartins are common, and the genitals with micro-sections of testis and epididymis are shown.

Breeders of pure breeds of goats in England have been concerned that even in single kids they got caprine freemartins. Such a single kid is shown with genitals and micro-section of ovi-testis. The 'clenses' of male and female twin goats show that there is no communication between the blood vessels.

Twin membranes of sheep show that there is no anastomosis between the blood

vessels of the opposite sides of the membranes, and there are no freemartins amongst sheep.

Professor HAMILTON asked whether these cases of single pregnancies in the goat could not be regarded simply as hermaphrodite. The term 'freemartin', he understood, meant a female co-twinned with a male.

Professor LOW replied that he thought, undoubtedly, they were not 'freemartins' and could be regarded as hermaphrodite. 'Hermaphrodite' is a term that may be applied to 'freemartins' and he often talked of 'freemartin' pigs as being hermaphrodite.

Professor J. P. HILL said that he had recently noted the presence of a definite anastomosis between the placental vessels of the two foetuses in a twin pregnancy in a marmoset.

Dr R. G. HARRISON remarked that the existence of specimens of this nature in the Glasgow Hunterian Museum was evidence that William Hunter shared his brother's interest in this matter.

DEMONSTRATIONS

London (Royal Free Hospital) School of Medicine for Women:

(1) M. F. LUCAS KEENE: Sections of Human Embryos (6·5 mm., 10 mm., 15 mm. and 18 mm.) illustrating development in the pharyngeal region.

(2) E. E. HEWER: Some unusual histological preparations of human material.

(3) R. O'RAHILLY: The radiological anatomy of the carpus of Colobus.

(4) R. E. M. BOWDEN: Dissections of foetal limbs.

JUNE 1947

The Summer Meeting of the Society, for the Session 1946–47, was held in the Department of Anatomy, University New Buildings, Teviot Place, Edinburgh, on Friday and Saturday, 27 and 28 June 1947, at 9.15 a.m. each day, the President (Professor J. C. BRASH) in the Chair.

Friday, 27 June

Professor G. A. G. MITCHELL read a paper on 'An anatomical evaluation of operations for hypertension'.

Accepting authoritative views on the essentials in sympathectomies for hypertension as a basis for evaluation, it was shown that the Peet and the Allen-Adson procedures, and even the Smithwick operation, were insufficiently extensive to produce the desired results in all cases, because they might miss sympathetic fibres passing to the abdominal splanchnic area via aortic and oesophageal filaments arising from the splanchnic nerves and sympathetic trunks above the level of section, by the para-aortic nerves, in the phrenics, or in the oesophageal plexus. It was also demonstrated that these operations might not interrupt renal contributions arising below the second lumbar ganglia, or communications between the renal and the intermesenteric or spermatic plexuses. While blunderbuss sympathectomies were unscientific, a more limited attack was frustrated by our ignorance of the exact pathways concerned in the peripheral or central nervous systems, so, accepting the premise about the basic essentials, on anatomical grounds it was suggested that the optimum sympathectomy would be bilateral resection of the sympathetic trunks from the fourth thoracic to the third lumbar ganglia and removal of the splanchnic nerves.

Professor YOFFEY inquired if Professor Mitchell had had the opportunity of examining the adrenal medulla of cases following resection of the sympathetic.

Professor MITCHELL replied that he had not personally examined the suprarenal gland after operation for hypertension, but a group of American workers was presently investigating this problem.

Dr RICHARDS (representing Professor LEARMOUTH) said that he had recently visited various clinics in America where he had seen many operations for hypertension. Professors White and Smithwick in America, and Learmouth in Edinburgh, were now employing a more extensive operation, very similar in extent to the one advocated by Professor Mitchell.

Dr GLEES asked about the time vasodilatation persisted after operation and Professor MITCHELL replied that the periods varied, doubtless depending on individual factors and the extent of the denervation. In adequate operations it was sufficiently permanent to produce the desired clinical results.

Dr G. WEDDELL, for Dr D. C. SINCLAIR, read a paper entitled 'Further observations on the sensory changes induced by a compression cuff on a human limb'.

Lewis, Pickering & Rothschild (1931) describe a regularly ascending 'centripetal sensory paralysis' occurring in the periphery following the inflation of a sphygmomanometer cuff on a human arm at a pressure above systolic blood pressure. They attribute the mode of spread of anaesthesia to an increasing susceptibility of individual nerve fibres to the effects of pressure as they are traced proximally from the periphery.

We have confirmed that anaesthesia is broadly speaking 'centripetal' in that touch sensibility is lost in the hand sooner than in the forearm. Nevertheless, investigation of the progress of anaesthesia within the territory of any one nerve fails to confirm any regular centripetal spread. There is thus evidence that the pressure of the cuff selectively affects different nerve trunks but not the individual touch fibres within each trunk.

The hypothesis of Lewis *et al.* implies that the more proximally the cuff is situated the sooner will anaesthesia appear in the periphery. We have found that an alteration of 3 cm. in the position of the cuff, if made in the region of the elbow, can alter the time of onset of anaesthesia considerably. Above the elbow, however, an alteration of as much as 23 cm. may be without effect. It follows that no regular sensitivity 'gradient' such as that suggested by Lewis *et al.* can exist.

It is submitted that the findings can be explained by the following anatomical considerations: (1) The position of the main nerve trunks render them more vulnerable to encircling pressure in the arm than in the forearm. (2) The nerves supplying the front of the forearm are less readily compressed in the arm than those supplying the hand, by reason of their different anatomical relationships.

Professor KIRK inquired if observations were made on the radial nerve and Dr WEDDELL replied that this nerve had also been investigated and the results were in accord with those obtained for other nerves.

Dr WHILLIS, who asked if stimulation of deeper nerves had been carried out, was informed that only the purely cutaneous nerves were studied in detail and no definite statement could be made with regard to deeply seated nerves.

Dr W. H. FEINDEL read a paper on 'The significance of multiple innervation of cutaneous pain "spots" in relation to the quality of pain sensibility. I. Physiological observations.'

The association of qualitative changes in pain sensibility with 'hypoaesthesia' has been frequently noted since the studies of Head (1905), but no satisfactory explanation of the phenomenon has been put forward.

A convenient method of producing a progressive loss of cutaneous sensibility is by means of a sphygmomanometer cuff applied to the arm at a tension exceeding systolic blood pressure. In such compression experiments it has been shown by Zotterman (1933) that for a given sensory modality there is a progressive decrease in the number of functional nerve fibres. It has also been observed that at a certain stage in this process alteration in the quality of pain sensibility occurs. We have found that the

pain aroused by pinprick under such circumstances is interpreted subjectively as an unpleasant stinging sensation. Although the intensity of this sensation may be small it engenders a feeling of emotional tension which usually leads to a generalized withdrawal reaction. It is in fact similar to the pain described by Head as protopathic.

If the all-or-none law of nerve conduction holds for pain sensibility in man this alteration in quality of sensation can only be related to factors other than changes taking place in a single nerve fibre. It is suggested that the qualitative change is, in fact, related to a disturbance of the normal pattern of multiple innervation of cutaneous sensory 'spots'.

Dr G. WEDDELL read a paper on 'The significance of multiple innervation of cutaneous pain "spots" in relation to the quality of pain sensibility. II. Histological observations.'

It is well known that pain of an unpleasant quality can be elicited from certain cutaneous scars, and also from skin at the borders of areas which have either been recently denervated or are in the course of sensory recovery. Sensory tests have been carried out in several such cases, and the areas of skin concerned have subsequently been examined histologically after vital staining with methylene blue. It was found that in all cases where pain of an unpleasant quality could be aroused the nerve nets and terminals subserving pain were invariably isolated from their neighbours, in contrast to the interweaving which normally obtains.

On the other hand, the threshold recorded was found to be correlated with abnormalities in the morphology of the endings. The lowest thresholds occurred when the subjacent pain fibres were surmounted by growth cones ending just beneath the basal layer of the epidermis.

Direct stimulation of pain fibres in a human digital nerve has shown conclusively that such fibres obey the 'all or none' law. It follows that no modification of the characteristics of the stimulus applied to a single pain fibre will give rise to any change in the quality of the sensation perceived. From the observations reported above, it is suggested that changes in quality are due to alteration of the number and peripheral pattern of fibres involved in the conduction of impulses arising from a given stimulus.

Professor Dow asked if Dr Weddell's observations agreed with those of the late Professor Waterston that the nerves of the epithelium are the nerves of light tactile sensation and that they do not under ordinary conditions convey impulses which give rise to pain: sensibility to pain on the other hand being subserved by the corium.

Dr WEDDELL replied that his findings were in harmony with those of Waterston.

Professor WEST inquired about the early effects of cuff pressure and Dr WEDDELL answered that up to systolic pressure 'compression tingling' is produced; this passes off and is followed by dulled touch sensation for a period of anything up to 35 min. The nerve fibres fall out in random order.

Dr REWELL asked if the cuff pressure was on the nerve, or on the blood supply to the nerve and was told that it was exerted on both. Dr REWELL then went on to say that like all observers who have applied tourniquets to limbs for any length of time, he had noticed the change in the character of the sensation produced by touch stimuli and he agreed that it becomes more intense and generalized and evidently of the protopathic type. In the leg, he found the onset of the change-over to this type of sensation took much longer to happen and it persisted for a much longer period before anaesthesia set in. This picture is complicated by the fact that the prolonged application of a tourniquet to the leg gives rise to a marked feeling of general discomfort which limits the duration of any experiment. Even when the ischaemic limb is exercised, this feeling demands removal of the tourniquet before the onset of muscle cramps.

Dr A. Mohuiddin read a paper entitled 'The vomeronasal nerve and the nervus terminalis in the human embryo'.

The vomeronasal nerve and the nervus terminalis were examined in an embryo of 125 mm. crown-rump length from Dr Hewer's collection and a model was prepared. The vomeronasal organ was found to be well developed. The vomeronasal nerves were seen to be in a single large bundle on the right and two bundles on the left, and were lodged in grooves which deepened from below upwards. The accessory olfactory formation was recognizable as a distinct bulging on the dorsomedial aspect of the lfactory crus.

Anterior to the vomeronasal nerve several smaller bundles of nerves were seen; one of them was identified as a part of the nervus terminalis and others were probably also parts of it. The nervus terminalis was found to run upwards almost parallel to the vomeronasal nerve and entered the skull through the anterior part of lamina cribrosa. A well-defined ganglion terminalis was seen on it lying medial to the olfactory bulb. Scattered ganglion cells were also found on the intranasal course of the nervus terminalis. There was no evidence of the fibres of the nervus terminalis being incorporated in the vomeronasal bundles; it was seen entering the olfactory crus over a relatively wide area a little behind the accessory olfactory formation. No fibres were seen joining the infero-medial aspect of the hemisphere as has been stated by some investigators.

Dr Brandt remarked that there are three types of nerves which supply the vomeronasal organ, viz. the olfactory nerve, the nervus terminalis and the trigeminal and he inquired whether Dr Mohuiddin had been able to trace these in his silver preparations of human material.

Dr Mohuiddin replied that he had been able to do so but that the nerve endings were not specifically demonstrated.

Dr A. Brodal (introduced by Dr G. Weddell) read a paper on 'Experimental investigations of fibre connexions of the olfactory system'.

As part of a research programme on the olfactory system started in the Department of Anatomy, Oxford, an attempt has been made to trace the origin of some fibre tracts of this system by studying the retrograde changes appearing in nerve cells after their axons have been cut. The modified Gudden method developed previously by the author was adopted in these studies, since in investigations on other fibre systems this has proved to be superior to the ordinary procedure in which adult animals are used.

The results of experiments in which the anterior commissure was transected in young rats (9–12 days old) were described. Retrograde cell changes were observed in the following structures after mid-line lesions of the anterior commissure; the dorsal part of the anterior olfactory nucleus, the nucleus of the lateral olfactory tract, the nucleus of the stria terminalis, the piriform cortex, the entorhinal area, the medial and cortical amygdaloid nuclei and in parts of the neopallial cortex. The changes were bilateral and approximately of the same intensity on both sides. These changes occurred also in cases in which the commissure was transected lateral to the mid-line. There was never a total affection of all cells in the structures mentioned.

It is concluded that the nuclei showing changes in these experiments are the origin of fibres of the anterior commissure. Whether the fibres are all truly commissural or only crossing, cannot be decided. All of these nuclei have other efferent connexions in addition to those crossing in the commissure.

The findings described substantiate conclusions drawn from studies of normal material. Only with regard to the amygdaloid nucleus are they at variance, since workers on normal material have maintained that the amygdaloid fibres to the commissure are derived mainly from the lateral and partly from the basal amygdaloid nuclei. These did not present convincing changes in these experiments.

Professor GOLDBY asked whether there was any concomitant injury to the septal areas in the experiments, and whether such lesions might be responsible for the changes observed.

Dr BRODAL, in his reply, mentioned that it is virtually impossible to transect the anterior commissure without inflicting some damage to the septal areas. However, the bilateral symmetrical changes can only be explained as being due to a lesion of fibres crossing in the commissure, since by any other arrangement of the fibres the changes would have been asymmetrical. Such changes were, in fact, observed in two cases with unilateral lesions, and allowed of some conclusions concerning connexions of the septal area.

Dr P. GLEES read a paper entitled 'Experimental study of the pyramidal tract'.

The origin of the pyramidal tract and its composition at medullary level has been disputed in recent years. Not only the motor cortex but adjacent parts of the cerebral cortex are said to contribute to the cortico-spinal tract. In addition it has been shown by several workers that the numbers of the giant cells of Betz in the motor cortex are not in proportion to the numbers of pyramidal fibres. It appears likely, therefore, that other cells of the fifth layer of the motor cortex make their contribution by sending their axons into the spinal cord, and it is also possible that other cortical layers are efferent. It has recently been claimed that the pyramid at medullary level contains not only cortico-spinal efferents but other fibre systems as well.

Material used by Professor Liddell and Dr Phillips for their physiological experiments on the pyramidal system of cats was investigated histologically and in addition a number of experiments on rabbits, cats and monkeys, causing either total ablation of the motor area, or small lesions were performed.

The number of normal fibres in the pyramid at medullary level was counted in myelin and silver preparations, and the fibre losses occurring after small and large cortical lesions were assessed at various levels. These histological observations showed that the greater part, if not all, of the cortico-spinal fibres originate in the motor area itself, and that the pyramid at medullary level is entirely cortical in origin. There is, however, a zone beneath the inferior olive which remains intact following ablation of the motor cortex and the data indicate that the fibres in this region belong to the medial fillet.

The mode of degeneration of the cortico-spinal fibres was studied by means of Marchi and silver preparations in relation to the time factor and it was demonstrated that the Marchi positive staining appears on the third day after injury and that axonal changes occur at the same time.

Dr ROMANES remarked on the difference in size of the nerve fibres in early and late degeneration and inquired about the nature of the degeneration following lesions of the motor cortex in the monkey.

Dr GLEES replied that a recent continuation of this work in the monkey showed that very small lesions in the motor area produced no concentrated Marchi degeneration confined to a definite area in the pyramid, but a scattered degeneration indicating that fibres from the subdivisions of the motor cortex intermingle with each other in their descending course.

Dr G. J. ROMANES read a paper entitled 'Notes on the spinal cord in acephalic sheep foetuses'.

The general form of one, and the spinal cords from two, acephalic sheep foetuses were demonstrated. The spinal cords consisted principally of thoracic and lumbosacral segments and showed an obliquity of the spinal roots commensurate with a relative shrinkage of the cords towards their centres. Internally the cords showed a normal, or slightly increased, amount of white and grey matter without evidence of any loss of cells or fibres, except in the thoracic nucleus which was devoid of cells.

It seemed likely that the number of cells in the anterior horn and posterior root ganglia of the lumbar enlargement was reduced as there were approximately 10% fewer medullated fibres in the nerves supplying the hind limbs than normal. This could be accounted for by the reduced size of the hind limbs.

It is concluded that in the sheep the lower half of the spinal cord can develop normally in the absence of the brain and presumably receives few descending tracts of any size from it.

Professor BOYD inquired about the nature and amount of the somatic musculature in the lower limbs, and Dr ROMANES replied that the muscle masses were all present but poorly differentiated and small. Each nerve could be recognized as a separate entity and followed a normal course in the limb.

Dr BRANDT remarked that the independence of differentiation of the embryonic spinal cord from the brain can be shown by the methods of experimental embryology. When the fore-limb rudiment in *Amblystoma* is excised the spinal ganglia undergo marked hypoplasia; when a supernumerary limb is transplanted the ganglia show hyperplasia. The motor centres of the cord respond in some amphibia and birds to peripheral changes. Growth and development of the spinal cord is correlated with the periphery rather than with the brain.

Dr W. F. HARPER read a paper entitled 'Observations on the blood vasculature of the turbinate mucosa in man and other mammals'.

In man the main arteries occupy an interosseous position in the turbinate bones and their branches pass off in several groups to form a fine subepithelial plexus. The latter opens into wide 'blood spaces' (veins) of irregular shape, which occupy the main mass of the tunica propria. In addition the mucosal arteries effect direct arterio-venous communications with the 'blood spaces'. The 'blood spaces' open into a periosteal plexus of veins and into a system of veins contained in bony canals in the turbinates. The structure of the walls of the 'blood spaces' and their arterio-venous connexions was detailed and discussed with special reference to the erectile capacity of the conchal mucosa.

In other mammals studied large venous spaces are also characteristic of the turbinate mucosa, but in their structure, pattern and connexions they present fundamental differences to that obtaining in man. In one order (ungulata) arterio-venous anastomoses between the mucosal arteries and the venous spaces were demonstrated.

Lack of time prevented discussion of this paper.

Dr E. J. FIELD presented a paper entitled 'Observations on the development of the conducting system in the sheep heart'.

A definite bundle of fibres straddling the muscular interventricular septum becomes apparent at about the 18 mm. stage of development, and has the form of an inverted U. When followed in serial sections at 8 μ the highest point of this formation is seen to possess no connexion with the atrial musculature, so that a true 'Bundle of His' cannot be said to exist. Later this apical region increases rather to produce a 'Node of Tawara'. The cells of the early bundle are swollen and clear with nuclei which often show some degree of pyknosis. Glycogen, as demonstrated by Best's carmine stain, seems rather more abundant in these cells than in the rest of the myocardium, but the picture obtained is variable.

Lining the ventricular cavities is a meshwork of clear embryonic-looking cells which at this stage have more of the appearance of adult Purkinje cells. It seems that as the compact myocardium thickens, the inner basket layer trabeculae may be caught up within it and so develop into the Purkinje system of the adult heart. Many of the 'vessels' described as accompanying the system may be of the nature of crypts and produced in the same manner.

Dr ROMANES suggested that Lison's method for staining glycogen would probably give better results than Best's carmine stain.

Dr WALLS congratulated Dr Field on the sections which he had shown, especially those in which the glycogen content of the bundle was so well marked. He did not think, however, that Dr Field was justified in querying the conducting function of the system because of the peculiarities in appearance of certain fibres; in the human bundle long stretches conducted very well in the absence of large typical Purkinje fibres.

Dr FIELD replied that the evidence for the conducting function of the atrio-ventricular bundle is not really conclusive. It is a kind of *idée fixé* which has existed for the past 25 years.

Dr WALLS, continuing, remarked that, morphological considerations apart, was the tremendous weight of experimental evidence built up by Sir Thomas Lewis and others then to be ignored?

Drs J. B. BRIERLEY and E. J. FIELD read a paper on 'The connexions of the sub-arachnoid space with the lymphatic system'.

Although the existence of a connexion between the cranial sub-arachnoid space and the cervical lymphatics via the olfactory nerve sheath and the nasal mucosa has long been recognized, little attention has been paid to the connexions of the spinal sub-arachnoid space.

Evidence is now presented to show that a segmental sub-arachnoid-lymphatic connexion exists throughout the length of the cord. Filtered Chinese ink, prepared by rubbing up in saline, was introduced into the lateral ventricles of living rabbits under carefully controlled physiological pressures and with precautions against leakage. The amount of Chinese ink introduced was always rather less than the amount of cerebro-spinal fluid previously withdrawn. In addition to the anticipated filling of the deep and superficial cervical lymph nodes, there was macroscopic filling of segmentally arranged lymph glands along the posterior thoracic and abdominal walls, and a particularly marked filling of the glands along the iliac vessels and in the hollow of the sacrum. After a single intra-ventricular injection of 1·0 c.c. of ink, maximal filling of these glands was attained at the end of about 60 hr.

The precise pathway from the sub-arachnoid space to the individual lymph nodes is still being traced out and demands a technique that will completely fill the intervening lymph vessels with ink.

Dr WEDDELL asked whether the spleens of the experimental animals had been examined and was informed that they had not.

Dr P. BACSICH asked when Speransky published his observations and was told that his papers appeared in 1927, 1928 and 1929. Dr BACSICH then remarked that Lenhossek, in his text-book of Anatomy (1923), had already called attention to the rapid appearance in the cervical and thoracic lymph nodes of coloured substances injected into the sub-arachnoid space of experimental animals. He also mentioned the possibility of a drainage via the cranial and spinal nerves, and warned that increased pressure used during injection might lead to erroneous conclusions.

Dr BRIERLEY, in reply, stated that there was no indication of passage of the injection mass along nerves if the pressure is carefully regulated.

Drs A. SLESSOR and G. M. WYBURN on 'Bone formation'.

The occurrence of bone formation in the host tissues (guinea-pig) following the presence of bone homografts in the subcutaneous tissues for 5–6 days was previously reported (Wyburn and Bacsich). Bone homografts consisting of the femoral epiphysis (bone and cartilage) were implanted and maintained in the subcutaneous tissues of guinea-pigs for 3 weeks. There was no evidence of bone formation by the tissues of the host.

Bone homografts (pieces of shaft of femur) were implanted into the flexor muscles of the hind limbs of rabbits. After 4 weeks there was no evidence of bone formation by the host tissues.

Blum (1944) asserts that he obtained formation of bone in rabbit muscle after the intra-muscular injection of phosphatase. His experiments were repeated and, in addition, pellets of phosphatase were implanted intra-muscularly and left for 4–5 weeks. There was no evidence of bone formation.

Dr M. A. MacConaill on 'The kinetics of synovial joints'.

A synovial joint is a bursa which is a locus of tangential displacement of at least one pair of skeletal elements upon each other. There is now a corpus of both structural and experimental evidence that the lubrication of the joint-system is of the viscous film type. The present paper is intended to show that the usual complex treatment of the kinetical problems involved can be reduced to a form familiar to all biologists, and to involve no concepts other than those already demonstrated to hold for the behaviour of viscous-fluid systems in other fields of biophysics. In particular, the force (F) available for the thrust against a weight carried at the joint is shown to be:

$$F = \frac{LUAS}{H^2} \times a \text{ 'shape coefficient'},$$

L being the viscosity of synovia, U being the speed of motion, A the area of articulation, S the distance traversed, and H the thickness of the viscous film.

Owing to the absence of Drs Wyburn and MacConaill the above two papers were read by title only.

Drs D. V. Davies and D. A. W. Edwards read a paper entitled 'The blood supply of the synovial membrane and the semilunar discs of the knee'.

William Hunter's account of the circulus vasculosus articuli in 1743 remains the only description of the blood vascular channels in joint structures. Subsequent investigators have concerned themselves only with the superficial nature of the capillaries of the synovial membrane. The vascular pattern in the synovial membrane varies according to the type of membrane and consequently the region in the joint. The semilunar cartilages are vascularized only in their peripheral parts. The horns of the discs are better supplied with blood vessels than elsewhere. The blood vessels to the discs enter mainly at the horns and the capillary loops end at some distance from the free edge. The vascular patterns seen in these regions were described both in the new-born and adult human.

Dr Whillis remarked that it would be instructive to make a cast of the blood-vessel pattern in the disc canals, and Mr Jardine said that he was surprised at the amount of vascularity in the meniscus.

Drs H. D. Darcus, G. Weddell and J. S. Weiner read a paper illustrated by a cinematograph film in slow motion on 'Some observations on the efficiency of muscular action with respect to fatigue and oxygen consumption'.

Trials have been carried out to compare the efficiency of a new type of spade invented by Dr G. H. Bates of the Staffordshire Education Committee, against that of an ordinary spade of similar weight and with a blade of similar dimensions. It is claimed for the modified spade that for comparable amount of work fatigue is less, especially in relation to the sacro-spinalis muscles.

One subject was used and the oxygen consumption for a given amount of work with each spade was measured. An attempt was made to correlate the findings with the movement of the subject as determined by slow motion cinematography.

Dr Whillis inquired if the respiration rates had been ascertained, and on being informed that they had not, he remarked that an investigation of this nature could not be regarded as complete without such information.

Dr I. W. Monie read a paper on 'The development of double ureters in human embryos'.

In fully developed double ureter it is usual to find that the ureter from the upper pole of the kidney opens into the bladder caudal and medial to the opening of the ureter draining the lower pole. This state of affairs is considered to be due to the cranial migration of the originally more caudally placed orifice of the ureter from the lower portion of the kidney.

Examination of a 40 mm. embryo with bilateral double ureter showed this cranial migration taking place and that the development was more advanced on the right side where both ureteric openings lay on the same horizontal plane. A 99 mm. embryo with unilateral double ureter, on the other hand, indicated that by this stage the ureteric openings had taken up their definitive positions in the bladder.

It is suggested that the alteration in positions of the ureteric openings is due partly to a diminution of the rate of growth of the trigonal region of the bladder which is marked in the earlier stages.

Professor Kirk questioned whether the medial ureteric orifice belonged to the primary ureteric bud and asked if the embryos under consideration showed this to be the case.

Dr Monie, in reply, said that authorities differed with regard to which orifice belonged to the primary ureter and that the embryos examined did not shed any light on this matter.

The President remarked that Dr Monie had been very fortunate in securing such interesting specimens.

Dr J. S. Baxter and Professor J. M. Yoffey read a paper on 'Post-natal development of the renal tubules in the rat, stained intravitam'.

The elimination of trypan blue by the renal tubules has been studied in thirty-six rats, from birth up to 28 days of age. The dye is found in the cytoplasm of the epithelial cells of the proximal convoluted tubules. In the new-born animal there is a peripheral nephrogenic zone of the renal cortex where the nephrons are still undifferentiated and where dye is not found. The proximal convoluted tubules deep to this show increasing amounts of trypan blue in their cell cytoplasm as one passes towards the medulla, that is, the earlier differentiated nephrons contain more of it. This arrangement persists until 12 days when the nephrogenic zone is becoming differentiated, and very fine dye droplets can be seen in the tubules just under the renal capsule. The ability of the tubular epithelium to segregate vital dye is associated with the acquisition by it of a brush border.

After this time further tubule differentiation takes place and by 28 days of age all the proximal convoluted tubules exhibit dye in their cytoplasm in similar amounts, the earlier 'staircase' effect having disappeared.

The significance of these observations was discussed, (*a*) in relation to the function of the kidney in the new-born, and (*b*) with regard to the rates of kidney and body growth in the post-natal period.

Professor Hamilton asked if the segregation of trypan blue in these experiments was in any way related to the vascularity, and Dr Baxter replied that the dye might be brought to the tubule cells either by the blood stream, or by the lumen of the tubules themselves, but that this did not in any way affect the interpretation of the results.

Dr D. V. Davies read a paper on 'A benign tumour of the placenta'.

The tumour was attached to the margin of an otherwise normal full-term human placenta and consisted of three solid red masses, the largest of which was the size of a hen's egg projecting into the uterine lumen from the chorion laeve. It was supplied by foetal blood vessels, mainly direct from the umbilical vessels. Histologically it was

an angioendothelioma of the capillary type with the solid endotheliomatous areas predominating. It was pointed out that in all probability the majority of placental tumours so far reported belonged to the haemangioblastomas, though the more solid endotheliomatous types had frequently been incorrectly labelled as sarcomata, fibrosarcomata and fibromata.

Dr WHILLIS asked why this tumour was considered of chorionic origin. Dr DAVIES, in his reply, pointed out that it was supplied by umbilical blood vessels, and that on its surface there were remnants of trophoblast indicating that the tumour originated within the trophoblast and secondarily extended into the uterine lumen, carrying portions of the trophoblast on its outer surface.

Mr F. ROLAND WILDE read a paper entitled 'The anatomy of the ano-rectal region: preliminary report'.

The investigation was stimulated by a difficulty in reconciling various accounts of the anatomy of the ano-rectal region with the appearances as seen in the living patient both clinically and at operation.

Vertical sections made from fresh adult material show the external sphincter in three divisions, separated from the internal sphincter by the descending longitudinal fibres of the rectum. The large size of the internal sphincter is striking and its lower border corresponds to the commencement of true skin in the anal canal. The longitudinal muscle coat becomes increasingly fibrous as it descends to terminate in a number of fibromuscular septa. The latter are attached in the following manner: (*a*) some traverse the bundles of the subcutaneous external sphincter to be attached to the skin at or beyond the anal verge, (*b*) others pass between the subcutaneous external, and the internal sphincters, forming the anal intermuscular septum, and (*c*) the remainder become continuous with the submucosa of the anal canal by turning round the distal border of the internal sphincter. These involuntary fibres in the termination of the longitudinal coat form a radiating sheet immediately beneath the skin to which Ellis gave the name musculus corrugator cutis ani.

The simple tubular glands typical of rectal mucous membrane were described. Hilton's line appears to be a colour contrast between the brown pigmented skin of the anal verge, and the bluish tinted mucosa above. As observed by Hilton, the line lies exactly at the junction of the ental and ectal sphincters. Intervening between the pectinate line and Hilton's line there is a zone of stratified epithelium some 10 mm. broad, devoid of dermal papillæ and skin appendages. This Stroud has called the 'pecten'. There is a definite layer of fibromuscular tissue in the submucosa of the pecten, continuous distally with the longitudinal muscle coat round the inferior border of the internal sphincter. Proximally these involuntary fibres are more numerous and form a thick muscularis mucosae the deeper bundles of which blend with those of the internal sphincter.

Dr WHILLIS said that he was able to confirm most of Mr Wilde's findings but suggested there was no such entity as the anal intermuscular septum.

Mr WILDE replied that the longitudinal muscle coat is attached distally by a series of fibromuscular septa in a radiating manner, to the skin of the anal canal down to and beyond the anal verge. A few of these, composed largely of connective tissue, pass between the subcutaneous external and the internal sphincters, forming the anal intermuscular septum, but these most certainly do not constitute the whole attachment of the longitudinal muscle coat.

Dr O'RAHILLY inquired whether the subcutaneous external sphincter was synonymous with corrugator cutis ani.

Mr WILDE answered that Ellis described the corrugator cutis ani as a radiating sheet of involuntary fibres immediately beneath the skin. It is true that in his description of the external sphincter Ellis omitted any reference to the subcutaneous

portion, that is, the part devoid of bony attachment. But the subcutaneous external sphincter is a voluntary muscle whose fibres are arranged in a circular manner, which clearly does not fit in with Ellis's account of the corrugator cutis ani.

Professor KIRK remarked that he was surprised no mention had been made of the levator ani muscle. He understood that the sphincter ani profundus is composed entirely of the pubo-rectalis from levator ani.

Mr WILDE replied that he could not confirm this and he had seen no record of it in the literature read to date.

Drs C. W. D. LEWIS and J. N. M. PARRY read a paper on 'A double aortic arch in man'.

The specimen was discovered during the routine dissection of a male subject aged 52 years. It showed a double aortic arch both anterior and posterior arches being widely patent and forming a ring enclosing the trachea and oesophagus. The posterior arch was the larger. Slides illustrating the probable course of development of the anomaly were shown. The rarity of the condition was noted and similar cases in the literature were referred to. A clinical syndrome of double aortic arch was described.

Dr INKSTER referred to three cases recorded in the *Proc. Roy. Acad. Med.* in Ireland (29 March 1946). Two were found by Dr Hardman in radiographs, and one came from the dissecting room of Trinity College, Dublin. In each case the arrangement of the vessels was rather similar to that of Lewis and Parry except that the anterior arch had not survived. The result was a 'seemingly' left arch passing behind the oesophagus. Since the true left arch had disappeared from in front of the oesophagus there were no symptoms referable to the condition.

Drs A. YOUNG and R. J. HARRISON read a paper on 'The anatomy of the retinacula of the wrist joint'.

Investigations were made on sixty dissections of the wrist and hand. The flexor retinaculum consists of three main portions, distinct anatomically, and possibly in function. The distal edge of each part is overlapped by the next more distal portion. Only the most distal is a true transverse carpal ligament, extending from the hook of the hamate to the palmar lip of the trapezium. The central portion, partly shrouded by a portion of the piso-hamate ligament which fans out radial-wise, is not attached to the pisiform but can be traced down to the dorsal lip of the trapezium, and to the capitate, triquetral and lunate bones on the ulnar side. The most proximal portion, continuous with the enveloping layer of deep fascia of the forearm, is likewise not attached to the pisiform, but to the triquetral and lunate. The statement that the piso-triquetral attachments can be completely severed without opening the carpal tunnel has been shown to be true and, moreover, it is possible to divide between the radial side of the piso-triquetral joint and the carpal tunnel without opening either.

The extensor retinaculum glides freely over, and is not attached to the head or to the styloid process of the ulna, nor to the ulnar collateral ligament of the wrist joint. A deep layer of transverse fibres restrains the extensor carpi ulnaris tendon in the ulnar groove, but is lax enough to allow it to rise out of the groove in certain movements, e.g. in ulnar deviation of the supinated hand.

Professor KIRK inquired about the relation of the ulnar neurovascular bundle to the three parts of the flexor retinaculum.

Dr YOUNG replied that the bundle lay superficial to all three parts but deep to the volar carpal ligament which is continuous around the lower end of the tendon of the flexor carpi ulnaris with the more proximal fibres of the extensor retinaculum.

Following the Society's Dinner in the University Union, Park Place, on Friday 27 June, the members and guests returned to the Department of Anatomy where Dr LEON STANSFIELD STONE, Bronson Professor of Comparative Anatomy, Yale

University School of Medicine, demonstrated the following two-coloured motion picture films. (Each 400 ft. × 16 mm.)

I. 'Return of vision and functional polarization in retinae of transplanted salamander eyes.'

This film showed a new method of examining the visual mechanism through special studies of transplanted adult salamander eyes where the sensory retina degenerates and then regenerates from the surviving pigment cells. Vision is shown to return four times in the same adult salamander eye repeatedly transplanted to new hosts. Eyes were successfully exchanged between salamanders of different species and also grafted with success after 7 days of refrigeration.

Normal visuomotor responses were demonstrated as the animal follows a lure approaching in all directions. The normal animal also automatically follows vertical black and white stripes on a drum rotating in one direction only (temporonasal).

If the grafted eye is normally oriented visuomotor responses are normal. If the eye is excised, rotated 180° and reimplanted, abnormal swimming reactions and reversed visuomotor responses result after the new retina and optic nerve regenerate. Dorsoventral and nasotemporal axes may be independently rotated 180° in a single eye by exchanging right and left eyes and properly orienting them. Only through the reversed quadrants of the regenerated retina are the visuomotor responses reversed. These abnormal visuomotor responses remained established in animals kept alive for 3½ years. By rotating these eyes *in situ* back to normal position without injury to retina and optic nerve, normal visuomotor responses were immediately established. These results have an important bearing on experiments dealing with learning in animals.

Many control experiments were shown and attention was called to a new method for studying visual acuity and colour vision.

II. 'Development of the pouch young opossum, *Didelphys virginiana*.'

The development of the pouch young of this North American marsupial was shown from the time of birth to about 70 days later, when they leave the pouch for the first time. At close range one can see their growth and activities at frequent intervals during this entire period when they remain attached to the nipples within the pouch.

The gestation period is 12–13 days and the young are about half an inch in length at birth. At this time the eye is in the same stage of development as that of a 5-weeks' old human embryo. Since many other embryonic characteristics also exist at birth this marsupial presents unusual opportunities for experimental studies on a mammalian embryo.

The film also showed the laboratory conditions under which the breeding colony is kept and studied.

Saturday, 28 *June*

Professor J. C. Brash read a paper on 'The laryngeal air-sacs of the orang-utan'.

'Minnie' the female orang-utan of the Edinburgh Zoological Park, died suddenly from haemorrhage from an abdominal (intraperitoneal) gestation. The air-sacs appeared to be unusually extensive, and the opportunity was taken to make a complete dissection. This was facilitated by preliminary filling with Cathcart's casting mixture (gelatin, glycerin, zinc oxide), and the whole system, right and left sides, was filled from one point, there being a communication between the two main sacs in the suprasternal region. The arrangement of the sacs is described, special note being made of the axillary extensions both behind the clavicle and in front of it through the pectoral muscles. The sacs in this animal are much more extensive than hitherto described in the female orang-utan.

Various hypothetical explanations of the functional nature of the laryngeal air-sacs of anthropoids are considered; and a new, tentative suggestion (supported by simple

experiment) is put forward for discussion—that the sacs may function as accessory respiratory bags for rebreathing.

Professor KIRK who asked whether the laryngeal air-sacs in anthropoids normally communicate with each other was informed that they do.

Dr ROMANES asked if there was any particular reason why an orang-utan should rebreath, and Professor BRASH said that while he knew of none for certain it might come into play in the act of climbing.

Professor WEST who asked about the mechanism of closure of the air-sacs was told that a special laryngeal musculature at the site of the opening was responsible.

Professor MITCHELL remarked on the intimate relationship of the air-sacs to the great vessels at the root of the neck, and inquired if they might function in preventing pressure at this site.

Professor BRASH replied that that would be a reasonable assumption.

Dr HARPER asked if the axillary extension of the air-sacs, unilateral in the case described, and covered by a fasciculus of the pectoralis major might be regarded as an abnormality.

Professor BRASH said that this was a normal relationship and that the portion of the pectoralis major muscle lying superficial to the extension of the sac could act by compressing it.

Dr S. MOTTERSHEAD read a paper entitled 'Some anatomical causes of pressure at the cervico-brachial outlet'.

The presence of abnormal ribs as a cause of pressure at the thoracic outlet is well known, and scalenus anterior, scalenus medius, scalenus minimus and costo-clavicular pressure may also produce symptoms.

Scalenus anterior can irritate the brachial plexus by pressing it against the ventral end of a cervical rib. The lowest trunk may rest on the sharp, falciform fold occasionally present (2%) at its costal insertion, or the plexus may be suspended on a tendinous V formed by scalenus anterior and medius, a condition present in 15% of dissections.

Traction on the arm causing bowstringing of the plexus over the medial tendinous edge of scalenus medius is responsible for many cases with symptoms over the whole arm.

Compression of the first thoracic nerve between scalenus minimus or the costo-vertebral ligament and the inner border of the first rib is another occasional cause.

A study of clavicular movements in normal individuals and in the cadaver shows that compression of the neurovascular bundle between clavicle and first rib can only occur under abnormal conditions. Narrowing of the costo-clavicular interval owing to an abnormal thoracic outlet or a malunited fracture of the clavicle may lead to costo-clavicular pressure during retraction and abduction of the shoulder. Depression of the shoulder causes the clavicle to move anteriorly, thus widening the costo-clavicular space.

No one single cause can account for the symptoms in all cases. Operation, when undertaken, should be of an exploratory character.

Dr WEDDELL remarked on the multiplicity of the causes of pressure at the cervico-brachial outlet. On account of this the emphasis is rightly laid on the exploratory type of operation, preferably under local anaesthesia, so that the patient can execute movements.

Dr W. BRANDT read a paper on 'Measurements and description of the external appearance of some structures in human twin embryos'.

The usual method of indicating the age of young human embryos is to refer to the crown-rump length. The exact correlation between this index, actual age, and morphogenetic development is, however, far from established. Research on twin

embryos, which have the same age, is the readiest method for finding out the amount of variation of growth in a certain stage of development.

The twin embryos dealt with in this paper were non-identical. The total length of one embryo was 17 mm., of the other, 18 mm. Both embryos showed the same differentiation of their hand- and foot-plates and the same position of their elbow and knee joints. There was an umbilical hernia present in both embryos, although this does not normally appear until the 20–25 mm. stage. In the 17 mm. embryo of Mall the first indication of the caecum was visible in the shape of a small round elevation and the caudal limb of the primary intestinal loop showing the caecum lying on the left side. In both embryos the caecum was an elongated structure of 1 mm. in length running parallel to the axis of the gut, and the caudal limb of the loop with the caecum lay cranially. The same phenomenon of accelerated development was visible in the external genitalia.

It follows that in these embryos the stage of development was much more advanced than that of the 17 or 18 mm. embryo usually described, and that in both the development of some structures is accelerated at the same rate.

On the basis of these observations it seems advisable to provide more than just the measurement of crown-rump length as an index of foetal age, and to include a description of the stage of development of different combined structures.

Dr Walls said that morphological criteria are much needed as the age in days or weeks is very approximate. Crown-rump length is also not dependable because of such variables as shrinkage before measuring (specimens often received in alcohol, etc.), and the personal factors in making the actual measurements. He had also noted, the above considerations apart, that embryos of the same age and/or size, often showed differences in the degree of development.

Dr Baxter remarked that an evaluation of the age of embryos relative to their development such as Dr Brandt had indicated was presently being prepared by Professor Streeter in America.

Professor West said that morphological data concerning embryonic age were certainly most desirable, but one cannot always sacrifice the specimen for this purpose.

Dr A. G. H. Mitchinson and Professor J. M. Yoffey read a paper entitled 'Movements of the vocal cords and neighbouring structures in altering the pitch of the voice'.

In twenty-four subjects of both sexes and various ages the larynx was X-rayed during the humming of low and high notes. Humming was employed rather than singing in order to avoid the complicating factor of jaw movement. Elongation of the vocal cords in singing a high note can readily be demonstrated radiographically, as also rotation of the larynx downwards and forwards. But the larynx as a whole rises on high notes, and is displaced forwards, together with the hyoid bone. The trachea is elongated, as seen in a lateral X-ray and may undergo a characteristic change in outline in its upper end.

Professor Mitchell said that he doubted if a correct and full interpretation could be arrived at by fixing the lower jaw.

Professor Yoffey added that an attempt would be made to trace the movements by means of cinematography.

Dr W. C. Osman Hill read a paper on 'Adrenals of Capuchin monkeys'.

Several observers have noted large adrenals in platyrrhine monkeys. Some (e.g. Kølmer) have imputed this to medulla; others (e.g. Fox) have assumed it to be pathological. There is no evidence of its being pathological and the constancy of its occurrence renders this unlikely.

Examination of glands from ten *Cebus* monkeys varying from 3 weeks' old to full maturity has provided data for a tentative explanation of the hypertrophy as due mainly to deeper elements of the cortex. In view of the scarcity of anatomical material of the group, results seem worth recording pending accumulation of more complete series of wild-shot animals.

In *Cebus* adults the glands average 1/9 kidney weight (cf. 1/15 in a large catarrhine, 1/14 in lower mammals and 1/30 in man). Variations occur in relation to age, sex and species. Contrary to findings in Old World Primates, the organs are not unduly large in the new-born, though a small quantity of androgenic tissue (=foetal cortex, X-zone) is present. Presumably more is present in foetal life, but earlier degeneration is inferred to be correlated with rapid post-natal bodily maturation reported in capuchins.

Histologically, medulla is well developed, but tissue most in evidence is zona fasciculata, whilst in females some androgenic tissue persists. Zona glomerulosa is poorly differentiated at all ages, though the deeper layer of the capsule is very cellular. Zona reticularis varies individually, sexually and with age. Lacking at 3 weeks, it occurs at 1 year, though feebly in males. In adult male of *C. apella* it is lost, but traces occur in *C. xanthosternos*. In mature females it persists alongside androgenic tissue.

Professor YOFFEY inquired concerning the possibility of the zona glomerulosa giving rise to the zona fasciculata and Dr HILL replied that as far as *Cebus* was concerned the glomerulosa was so poorly differentiated that little evidence was presented for the theory in question, but that the appearances seen in the deep cellular layer of the capsule certainly admitted of the interpretation that the glomerulosa might be budded off therefrom.

Dr BAXTER said that the term 'androgenic tissue' was perhaps not the best one to employ—he preferred the term 'X-zone'.

Dr HILL admitted that he had borrowed the term from American literature and that he considered it inappropriate.

Professor J. M. YOFFEY and Dr J. S. BAXTER read a paper on 'Changes in the suprarenal gland of the rat following the administration of pituitary adrenotropic hormone and extract of suprarenal cortex'.

The suprarenal glands of adult male rats have been studied after intravenous or subcutaneous injection of pituitary adrenotropic hormone (Cortrophin—Organon) and extract of suprarenal cortex (Eschatin—Parke Davis and Co.). Sections were studied by means of the phenylhydrazine, plasmal and Schultz reactions, and staining with sudan black; the distribution of birefringent material was also observed.

Following the administration of eschatin, the lipoid content of the gland increases and the subglomerular zone tends to disappear. The accumulation of lipoid is very marked in the zona glomerulosa. After cortrophin the lipoid content first diminishes, then increases. The phenylhydrazine coloration becomes more intense and widespread, involving almost the entire cortex, while at the same time the zone in which the plasmal reaction occurs seems to become narrower. The possible significance of the results was discussed.

Professor WEST commented on the beauty of the specimens.

Dr R. G. HARRISON remarked that the lipin content of the rat adrenal, as he had observed it, seemed to be correlated with the histochemical changes found by Yoffey and Baxter. He considered that there was wider variation between the adrenals of normal adult rats than in immature animals.

Professor YOFFEY agreed that the use of immature animals might, in certain cases, be desirable.

Dr G. W. Harris mentioned the work of Miss Vogt on adrenalin stimulation of the cortex and asked if any evidence of such changes had been observed.

Professor Yoffey replied that no evidence had been obtained to support Miss Vogt's views.

Dr J. S. Baxter and Professor J. M. Yoffey read a paper entitled 'Histochemistry of the suprarenal cortex in man and the chimpanzee'.

A number of histochemical tests have been applied to the suprarenal cortex of man and the chimpanzee. The distribution of the following has been determined: (*a*) sudanophil lipoid; (*b*) birefringent material; (*c*) substances reacting with dinitrophenylhydrazine sulphate (DNPH—SO_4); (*d*) substances giving the 'plasmal reaction'; (*e*) lipines; (*f*) cholesterol. The significance of the observations was briefly discussed.

Dr Romanes asked how far the differences observed could be correlated with species difference and with changes in the cortex during pregnancy.

Dr Baxter replied that undoubtedly the fact that the chimpanzee adrenal was from an animal in the later stages of pregnancy had a most important bearing on the results. In that specimen there was cortical hypertrophy with depletion of the biologically active substances. No information was available on species differences.

Dr Glees inquired about the orientation of birefringent substances in the cortical cells and Dr Baxter replied that in the human the cortical cells were packed full of birefringent material. In the chimpanzee, while there was less, no constant orientation of it to the capillaries was observed.

Dr J. Brander read a paper on 'Secretory pathways of the anterior lobe of the hypophysis cerebri'.

Current teaching in explanation of some syndromes and of morbid changes in the hypophysis itself was mentioned. New evidence was presented in support of the unorthodox suggestion made in 1932 (*J. Anat.* **66**, 202) that the anterior lobe may function, in part at least, as a tubulo-racemose gland, secreting to the cleft which persists throughout life and retains communications with the original lumina of pars media and pars tuberalis. Colloid contents of cleft are probably extruded into veins at the inferior extremity of the main cleft (which enter the inferior venous sinus) and at the tips of the pars tuberalis. Type and extent of media and tuberalis in the human hypophysis appear without significance for the function of the organ in individuals. Attention is directed to the course of the veins from the 'infundibular recess' of the posterior lobe which pass along the posterior wall of the cleft to the inferior venous sinus.

Dr G. W. Harris commented on the terminology used for the pars tuberalis, and on the nature of the colloid.

Dr Brander, in reply, said that the term 'pars tuberalis' was reserved by him for the secondary diverticula from the upper lateral angles of the main cleft. He agreed that the nature of the colloid was very obscure.

Dr G. W. Harris read a paper on 'The hypophysial portal vessels of the porpoise (*Phocaena phocaena*)'.

The pituitary glands of some animals (whale, porpoise, sea-cow, Indian elephant, armadillo and birds) possess a connective tissue septum between the neural lobe and pars intermedia, or pars distalis if the intermedia is lacking. It seemed possible that the usual vascular link between the median eminence of the tuber cinereum and pars distalis, viz. the hypophysial portal vessels, might be excluded by the presence of the septum in these forms. To investigate the possibility a male porpoise (*Phocaena phocaena*) weighing 136 lb., and 5 ft. in length, was injected with India ink through the vertebral arteries. Serial sections through the pituitary gland and surrounding

structures show that the porpoise possesses a well-developed hypophysial portal system of blood vessels, passing from the median eminence to the pars and zona tuberalis (anterior to the septum), and thus into the sinusoids of the pars distalis. It seems then that the presence of a septum does not necessarily exclude the possibility that the hypothalamus influences the activity of the pars distalis by humoral transmission through the portal vessels. In this respect it is of interest that the pars distalis of the porpoise has been found free of hypothalamico-hypophysial nerve fibres (Drager, 1944).

Dr GLEES inquired if colloid had ever been tested biologically.

Dr HARRIS could give no answer but Dr J. BRANDER remarked that any biological tests of colloid that have been made show that it corresponds in its effects more with posterior pituitary principle than with any known anterior lobe secretions.

Dr C. L. FOSTER read a paper on 'Some observations upon the Golgi elements of the anterior pituitary cells of normal and stilboestrol treated male rats using the sudan black technique'.

It was found possible to demonstrate the Golgi zones of anterior pituitary cells with sudan black. The position and general morphology of the Golgi bodies closely resembled that revealed by the usual osmium tetroxide techniques.

The Golgi zone was generally found to contain three sorts of element: (*a*) diffuse sudanophil material in which were embedded (*b*) clear vacuoles, and (*c*) strongly sudanophil bodies. The latter were often associated with the surfaces of the vacuoles, when they were in the form of (1) crescentic caps, (2) partial or complete rims of apparently uniform thickness, or (3) small granules. Sometimes there were granular sudanophil bodies unassociated with vacuoles as well as vacuoles unassociated with sudanophil material.

The effect of stilboestrol was to cause hypertrophy of the Golgi zones, and this was associated with (*a*) an increase in volume of the region of diffuse lipins, (*b*) an increase in vacuolation, and (*c*) a rather more variable increase in the amount of strongly sudanophil material.

The strong affinity of the Golgi area for sudan black suggests that it possesses a high lipin content.

Dr BAXTER commented on the clarity of the preparations and the simplicity of the method as compared with the standard silver and osmium techniques. He mentioned his own independent experiences with the method, which, however, had not yielded anything like the results obtained by Dr Foster.

Dr FOSTER replying mentioned some simple technical procedures which entirely did away with precipitation difficulties in the staining method.

Dr R. G. HARRISON drew attention to the fact, repeatedly stressed by Baker, that sudan black is not specific for lipins.

Dr FOSTER agreed.

DEMONSTRATIONS

Edinburgh Meeting:

(1) J. C. BRASH: (*a*) Laryngeal air-sacs of orang-utan. (*b*) Abdominal pregnancy in orang-utan. (*c*) Achondroplasic foetus.

(2) J. C. BRASH and W. C. OSMAN HILL: Seven specimens of sympodial foetuses.

(3) W. C. OSMAN HILL: Form and structure of the adrenals of capuchin monkeys.

(4) G. J. ROMANES: (*a*) Sections of spinal cords from acephalic sheep foetuses. (*b*) Brain, showing absence of corpus callosum. (*c*) A silver method for staining sections of nervous system. (*d*) Simple device for differential counting of nerve-fibres, leucocytes, etc.

(5) J. A. Ross (introduced by J. C. BRASH): Injected placenta of uniovular (?)

female triplets, showing arterial anastomoses. (By kind permission of Professor R. J. Kellar).

(6) R. McWHIRTER, J. S. SHEARER and P. AITKEN (introduced by J. C. BRASH): Series of radiographs of special anatomical interest.

(7) J. B. MACDOUGALL and D. R. DOW: Specimens of Sympus Dipus and Cyclops.

(8) G. W. HARRIS: The hypophysial portal vessels of the porpoise (*Phocaena phocaena*).

(9) F. R. WILDE: The anatomy of the ano-rectal region.

(10) J. BRANDER: Venous channels in relation to secretory pathways of anterior lobe of hypophysis cerebri.

(11) T. GRAHAME: (*a*) Celloidin corrosion preparations of mammalian kidneys. (*b*) Alizarin preparations of foetal mammals.

(12) W. F. HARPER: Observations on the blood vasculature of the turbinate mucosa in man and other mammals.

(13) R. O'RAHILLY: Radiographic reconstruction.

(14) A. G. MITCHINSON and J. M. YOFFEY: X-rays of the larynx in humming low and high notes.

(15) J. S. BAXTER and J. M. YOFFEY: (*a*) Vital staining of the neonatal rat kidney. (*b*) Histochemical reactions in the suprarenal gland of chimpanzee and man.

(16) J. M. YOFFEY and J. S. BAXTER: Changes in the rat suprarenal following the administration of pituitary adrenotropic hormone and extract of suprarenal cortex.

(17) J. B. BRIERLEY and E. J. FIELD: Chinese ink-filled lymph glands in a rabbit following intraventricular injection of the ink.

(18) J. J. PRITCHARD and A. G. H. MENZIES: Acid phosphatase in the central nervous system.

(19) M. F. ASHLEY MONTAGU (introduced by G. WEDDELL): The upper jaw of a negro foetus showing the premaxillary element still partially separate, and its apical extremity exposed on the facial surface at the margin of the nasal aperture.

(20) D. GUTHRIE (introduced by J. C. BRASH): Selection of early works on Anatomy (by kind permission of Dr L. W. Sharp, University Librarian). Anatomical Museum, First Gallery.

(21) G. F. HAMILTON: Improved instrument for calculating the Lee-Pearson formulae for cranial capacity: constructed in the Department of Anatomy, University of Aberdeen.

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